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SESSION 1 JOINT OPENING SESSION

C1 ALS IN A WORLD OF MULTIPLE PHENOTYPES

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The last several decades have witnessed an exponential growth in the clinical phenotypes labelled as ALS, fuelled by the discovery of mutant genes, now numbering almost 30, which present as ALS. Each mutated gene appears to have a distinctive signature altering a complex pathway leading to motor neuron injury and death. Such pathways include compromise of cytoskeletal function and axonal transport, misfolded aggregated proteins and their impaired digestion by proteosomes and autophagy, and impaired RNA biology. Despite the diversity of molecular pathologies, different mutant genes can produce a single clinical phenotype, and a single genetic mutation can produce multiple clinical phenotypes. These familial ALS phenotypes are indistinguishable from sporadic ALS phenotypes which are also heterogeneous, with markedly variable sites of onset, ages of onset and rates of disease progression. More recently the heterogeneity of ALS has expanded with the recognition that approximately 50% of sporadic ALS patients posses the behavioural and cognitive impairment of frontotemporal dysfunction, and 15% manifest frank frontotemporal dementia. Thus ALS results from compromise of multiple molecular pathways and can present with multiple clinical phenotypes. An important goal is to identify factors that contribute to the diverse clinical expressions of this syndrome.

In affected members from a single family with the same mutation, age of onset and disease severity and duration can be quite variable. Thus other factors, possibly genetic or environmental, modify the expression of ALS. Potential factors can be identified with the use of transgenic mouse models of ALS, where neurons do not die alone; neuronal injury is non-cell-autonomous and depends on well-orchestrated dialogues involving motor neurons, glia and T cells. The immunological responses of glia and T cells are not merely the passive consequences of injury, but actively influence and significantly contribute to the balance of neuroprotection and neurotoxicity and thereby mediate neuronal injury and neuronal viability. Regardless whether mutations in a specific gene initiate a cascade of intraneuronal injury in inherited disease, or whether the cause of the initial neuronal injury is undefined as in sporadic disease, a similar inflammatory response ensues. In both inherited and sporadic cases of ALS, differing temporal and mechanistic compromise of intraneuronal organelles may contribute to variations in immune responsiveness and disease progression. Thus the innate and adaptive immune systems contribute to phenotypic heterogeneity and represent potentially important therapeutic targets for modifying expression of disease.

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SESSION 2A NEURONAL VULNERABILITY IN ALS/MND

C2   MECHANISMS UNDERLYING SELECTIVE NEURONAL VULNERABILITY IN ALS

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Keywords: excitability, ER stress, endogenous neuroprotection

Delaying clinical disease onset would greatly reduce the burden of neurodegenerative diseases, but the mechanisms that influence early preclinical progression are poorly understood. Here we show that in mouse models of familial motor neuron (MN) disease SOD1 mutants specifically render vulnerable MNs dependent on endogenous neuroprotection signaling involving excitability and mTOR. The most vulnerable low-excitability FF MNs already exhibited evidence of pathology and endogenous neuroprotection recruitment early postnatally. Enhancing MN excitability promoted MN neuroprotection and reversed misfolded SOD1 (misfSOD1) accumulation and MN pathology, whereas reducing MN excitability augmented misfSOD1 accumulation and accelerated disease. Modulating excitability and/or alpha-MN mTOR activity had comparable effects on the progression rates of motor dysfunction, denervation, and death. Therefore, excitability and mTOR are key endogenous neuroprotection mechanisms in motor neurons to counteract clinically important disease progression in ALS.

C3   SIZE-DEPENDENT AXON LOSS IN THE CORTICOSPINAL TRACT IN ALS PATIENTS WITH UPPER MOTOR NEURON SIGNS

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Keywords: size dependent axon loss, corticospinal tract, upper motor neuron

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that selectively involves both upper and lower motor neurons (UMN and LMN). The mechanism by which both systems are involved is unknown. However, a common pathological finding in the spinal cord is corticospinal tract (CST) degeneration. We recently found a combination of myelin loss together with axonal loss in the tract in patients (1). Interestingly, axons of different diameters are also affected.

Objectives: In order to better understand the pathology, we compared the degree of axonal degeneration in the CSTs in the presence or absence of UMN symptoms in well-characterized postmortem cervical, thoracic, and lumbar spinal cords regions to determine the anatomical relationships of the connection between upper and lower motor neuron systems in ALS patients.

Methods: We systemically observed myelin loss in three regions of spinal cord. The degree of loss of axons of different diameters was measured and quantified in the lateral and ventral CSTs and dorsal column regions in the different levels of spinal cords from ALS patients with and without clinical UMN symptoms as well as from control patients.

Results: Only patients with clinical upper neuron symptoms showed lateral and ventral CSTs degeneration (myelin and axon losses). We found that all three spinal cord regions were affected in the lateral CSTs but mainly two regions were affected in the ventral CSTs. Quantification of axon density showed a loss of axons of both small and large diameters in the lateral and ventral CSTs in ALS patients with UMN signs. Comparing to ALS patients without UMN signs, we see a unique gradient of smaller diameter axon loss from lumbar to thoracic and cervical in ALS patients with UMN sign. In ALS patients without UMN signs, we only see some loss of larger diameter axons in the lateral CST at all three levels.

Discussion and conclusion: Our current findings (1) show an 'All or None' effect of myelin–axon loss at all spinal cord levels of lateral CST and (2) appear to be a 'Dying Back' of only small but not large diameter axons in patients with UMN signs. Our results suggest that selective vulnerability of axonal loss depends on fiber size and should be considered in interpretation of pathology of corticospinal tracts in ALS patients with UMN signs. We hypothesize that aberrant glio-axonal interactions in the spinal cord could contribute into this process.

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C4   AXON DEGENERATION AND AXON PROTECTION IN ALS

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Background: Axon degeneration is a key pathological feature of ALS although the cause remains uncertain. Studies have shown that axon degeneration is a process separate from cell body apoptosis and protection from cell death may not prevent the axon from degenerating. In this respect, axon protection may be an attractive target for therapeutic intervention in ALS. Although mechanisms of axon degeneration following axon transection have been well characterized, little is known about mechanisms of axon degeneration in neurological disease and whether they share similarities with axonal transection. Our investigations have demonstrated that axon degeneration can result from excitotoxic insult in both cortical

and lower motor neurons grown in culture. Excitotoxicity is a key pathogenic process implicated in ALS leading us to propose that excitotoxic damage may contribute to axonal pathology in ALS.

Objectives: We have investigated axonopathy following excitotoxin exposure. Our focus has been the role of caspase activation, the cytoskeleton and the therapeutic potential of cytoskeletal stabilization to prevent axon degeneration. Current studies include development of animal models of excitotoxin induced axon degeneration.

Methods: Cortical neurons were cultured from C57Bl/6 mice and grown in compartmented microfluidic chambers to examine the role of axon and soma. Neurons at 10 days were exposed to 100μM kainic acid (18hours) in the absence or presence of taxol in the axon or soma compartment. Axonal fragmentation was determined from phase contrast images of axons. Immunohistochemical analysis was performed using antibodies to active caspase-3, neurofilament and MAP2. (n = 5 repeats from three separate cultures). To determine if excitotoxin induced axon degeneration shares mechanisms with axonal transection, cultured neurons were transfected with a construct that encodes the Wlds fusion protein (a gift from Michael Coleman), which protects axons from degeneration induced by transection. Transfected neurons were exposed to 100μM kainic acid.

Results: Kainic acid applied to soma induced a 41.3% ($\pm$ 8.5 SEM) increase in axon degeneration in the axon compartment, which was associated with axonal caspase-3. Pretreatment of axonal and somal compartments with taxol reduced subsequent kainic acid-induced caspase activation and axonopathy, with a more marked effect following taxol applied to the axonal compartment ($12.2 \pm 2.3\%$ fragmentation, $p < 0.05$) as compared to the somal compartment ($25.8 \pm 5.2\%$ fragmentation). Expression of *Wlds* provided axonal protection from degeneration.

Conclusion: Excitotoxin induced axon degeneration, unlike axonal transection involves activation of caspases. However, the protective effect of the *Wlds* construct suggests that excitotoxin induced axonopathy and also shares some similarities with axon transection. These studies highlight the importance of investigating disease specific mechanisms of axon degeneration and suggest that microtubule stabilization may be a viable therapeutic option for ALS, either alone or in combination with other therapies.

C5 INHIBITORY LOSS OR DYSFUNCTION: A PRIMARY MECHANISM IN ALS?

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Background: In ALS, increased excitability of circuitry precedes motor neuron degeneration, suggesting that ALS results from disturbances in regulation of cell excitability. However, the mechanism of presymptomatic excitability remains unknown. There is strong clinical evidence, in both cortical and spinal regions, of reduced inhibition implicating this as a potential primary mechanism. We examined the motor and somatosensory cortex of SOD1[G93A] and non-transgenic mice for expression of interneuron-specific calcium binding and neuropeptide protein markers. Studies were also performed in human ALS and control brain tissue, investigating the presence of pathological changes within interneuron populations.

Objectives: To characterise the pathological alterations to cortical interneuron subpopulations in ALS mouse models compared to that present in ALS tissue.

Methods: Cortical tissue from presymptomatic (8 week) and end-stage (20 week) SOD1[G93A] and ALS human cortex were serially sectioned (40um), alongside age-matched controls, and immunohistochemically labelled with antibodies against calretinin (CR), parvalbumin (PV), somatostatin (SOM), Neuropeptide Y (NPY) and Vasoactive Intestinal protein (VIP). Qualitative analysis of cell soma appearance was performed alongside with quantitation of cell number and soma area. The presence of ubiquitinated and SOD protein inclusions was also considered. Analysis was performed on confocal images of supragranular and infragranular motor and somatosensory cortex lamina.

Results: Analysis of presymptomatic SOD1[G93A] animals found CR[+] interneurons to be unchanged (n = 4, 22.218 ± 3.607) relative to that of controls (n = 4, 27.660 ± 3.404), despite a 24% reduction in SOD1[G93A] CR^{+} cells in this region. Analysis of end-stage SOD1[G93A] cortical regions, however, found the number of CR[+] interneurons in the supragranular motor cortex lamina (I–IV) was significantly decreased ($P < 0.05$) by 37% (n = 4, 12.53 ± 2.137 SEM), as compared with wild type (n = 4, 19.59 ± 2.452 SEM) (Two-way ANOVA, multiple comparisons Bonferroni Test). Analysis of end-stage SOD1[G93A] NPY[+] interneuron numbers in the infragranular motor cortex (V–VI) identified a significant increase ($P < 0.05$) by 40% (11 ± 1.493 SEM), as compared with wild type (6.592 ± 0.879 SEM). Analysis of VIP labelling revealed a significant decrease in SOD1[G93A] area of labelling in the infragranular somatosensory cortex lamina (V–VI) by 54% (n = 4, 33.799 ± 6.80), as compared with wild type (n = 4, 73.243 ± 6.072).

Discussion and conclusion: These findings demonstrate pathological alterations to inhibition in the end-stage SOD1[G93A] mouse model of ALS, specifically implicating CR, NPY and VIP interneuron populations in cortical dysfunction. Furthermore, results indicate variable susceptibility of interneuron populations, with varying cortical regions and functional domains affected. This suggests specific populations may be differentially implicated in the disease. Ongoing investigations utilising human ALS tissue and other ALS transgenics will be important for further interpreting this data and determining whether inhibitory loss could be a potential mechanism underlying ALS.

C6 PERIPHERAL NERVOUS SYSTEM DYSFUNCTION IN A RAT MODEL AND IN HUMAN MOTOR NERVE BIOPSIES OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: peripheral nerve, motor nerve biopsy, magnetic resonance imaging

Background: Despite the fact that the loss of peripheral axons is a major cause of disability in amyotrophic lateral sclerosis and motor axons are involved in the pathogenesis of this disease, the peripheral nervous system (PNS) involvement in amyotrophic lateral sclerosis (ALS) has not been extensively studied so far.

Objectives: To characterize in detail the natural history of the PNS damage in a hSOD-1[G93A] ALS rat model using both *in vivo* and *ex vivo* readouts and compare such results with histopathological findings in diagnostic motor nerve biopsies from ALS patients.

Methods: Longitudinal magnetic resonance imaging, neurophysiological, and histological investigations were employed to monitor the extents of PNS damage in a hSOD-1[G93A] ALS rat model. Light microscope and immunocytochemical analysis of human motor nerve biopsies were performed.

Results: By *in vivo* magnetic resonance imaging, follow-up of the sciatic nerve allowed to define the imaging signature of the disease. Initial abnormalities within sciatic nerve were detected by an increase of T2 relaxation time, before symptom onset. In addition, diffusion magnetic resonance imaging acquired during disease course showed a progressive increase of mean diffusivity (MD) and radial diffusivity ($l_\perp$) within the sciatic nerve which was associated with reduction of fractional anisotropy (FA) at advanced stage of disease. Neurophysiological examination showed a gradual reduction of sciatic nerve distal compound motor action potential amplitude during disease course and concomitant signs of active denervation at needle examination. Histology showed early impairment of the blood-nerve barrier, endoneurial oedema and acute axonal degeneration associated with signs of neuroinflammation, such as a macrophage response in the motor nerve compartments, before the appearance of symptoms. Progressive axonal degeneration and motor nerve fiber loss were observed, correlating with changes in MRI and neurophysiological studies.

Histopathologic studies of human diagnostic motor nerve biopsies confirmed the presence of signs of acute axonal degeneration, associated with signs of neuroinflammation, predominantly represented by endoneurial macrophages and occasional epineurial inflammatory infiltrates.

Discussion and conclusion: The functional and morphological platform established here could be used to follow disease progression in the amyotrophic lateral sclerosis rat model and to evaluate possible therapies relevant to the disease. Moreover, the study of the peripheral nervous system involvement could also shed new lights in the ALS disease pathogenesis.

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SESSION 2B AUTONOMY AND QUALITY OF LIFE

C7   TO TEST OR NOT TO TEST, THAT IS THE QUESTION

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ALS physicians and those affected by the condition are faced with a series of difficult dilemmas generated to a large extent from advances in genetics over the past decade.

We know that some forms of ALS are familial, and that the condition is linked in some cases to frontotemporal dementia, and also to a wider range of brain disorders including schizophrenia, major psychosis and possibly suicidal behaviour.

Familial disease implies that some people with ALS harbour one or possibly more genes that have greatly increased their risk of developing ALS. In others, the condition has developed following exposure to certain environmental factors, the nature of which are unknown, that may have interacted with a more complex array of genetic factors, many of which have yet to be determined.

The current EFNS guidelines state that testing should only be performed in patients with a known family history of ALS, and following appropriate genetic counselling.

The first dilemma is to establish a universal definition of familial disease. Surprisingly, there is no consensus for this at present. Familial disease can be difficult to detect in small families, but the risks of two people in a family developing ALS by chance increases in larger kindreds.

The second dilemma is whether to perform any genetic testing, given that there is currently no treatment. A decision is then required as to whether to look for all known variants in all known genes, or for variants one gene at a time. This is important for three reasons. Firstly because reported variants may not always be truly pathogenic, secondly because there is evolving evidence that ALS may be 'oligogenic', implying that variants in different disease-associated genes occur with greater frequency that would be expected by chance in people with apparently familial ALS. And thirdly the frequency of pathogenic variants differs across populations. There is also a cost implication if many genes are to be tested at the same time.

The third dilemma relates to the implications of testing for other family members. There are major ethical and legal implications of genetic testing, as once a disease variant is identified in an affected family member, all other members of the family are at risk, and decisions must be made regarding testing asymptomatic relatives, and whether pre-natal testing should be available. This is important because there are no preventative treatments at present, and because the penetrance of various pathogenic genetic variants has not been adequately established.

Ultimately, genetic testing for ALS will need to be standardized by modifying existing guidelines established for other diseases, supported by ongoing research in clinical and genetic epidemiology and governed by a robust ethical framework.

C8   ALS CLINICS AND THE EMERGING CHALLENGE OF GENETICS: A WORLDWIDE SURVEY

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Background: As more ALS genes have been identified, and phenotypic variability has expanded, how best to address the genetics of the disease in the clinic has become increasingly complex.

Objective: To determine how ALS clinics in North America, Europe and Israel address the genetics of motor neuron disease.

Methods: We sent out emails to 203 ALS/MND clinics with a link to the survey web site (Survey Monkey.com).

Results: Eighty-seven responses were received, for a return rate of 43%. 81% of the respondents were in an academic setting. The majority did not have a genetic counsellor or geneticist (GC/G) in their clinic but could readily make an appointment locally (74.4%), 19.8% had a GC/G at selected clinics and 5.8% had them available at every clinic. Time to get an appointment with a GC/G was less than 2 months for 85.7%. Overwhelmingly, neurologists were willing to discuss and send genetic testing on patients without first getting a genetics consult (82.9%). The most common reasons for sending someone for a genetics consult were an ALS patient with a family history of ALS, and an ALS patient or their family member who specifically requests the referral. For patients with a family history of ALS (gene unknown), 30.3% test commercially for all genes available, 25% do selective testing commercially, 23.7% would do base testing on results of a genetics consult, 14.5% would test for research purposes only, and 6.6% would not do testing. When a pathological mutation is identified, 54.8% refer the patient for genetic counselling, the neurologist does the genetic counselling in 39.3%, and 25% refer family members for counselling. When recommendations are made to get genetic testing in patients with a family history of ALS, 33.7% find that none refuse, 47.0% report fewer than 1/3 refuse, 16.9% report between 1/3 and 2/3 refuse, and 2.4% report more than 2/3 refuse. Uncertainty about what positive results mean in the presence of a negative family history was listed as the first or second biggest challenge regarding genetic testing by 50% of respondents. However, among US neurologists, 74% chose cost as the first or second biggest challenge regarding genetic testing while this was true for only 13% of non-US neurologists.

Discussion and conclusion: Practice patterns regarding genetic testing vary widely in ALS clinics. This survey emphasizes the need for developing guidelines regarding such testing, though differences in insurance coverage need to be taken into account when these are developed.

C9   DEVELOPING A MODEL OF PATIENT-CENTRED DECISION-MAKING FOR AMYOTROPHIC LATERAL SCLEROSIS MULTIDISCIPLINARY CARE

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Keywords: patient decision-making, multidisciplinary care, decision-making model

Background: Amyotrophic lateral sclerosis (ALS) patients continually make decisions for symptom management and quality of life as their condition deteriorates. Health professionals aim to support patient autonomy through specialised ALS multidisciplinary care delivery. Even so, established models of patient-centred decision making do not account for the complex and changing needs of ALS patients throughout the disease trajectory.

Objectives: To investigate ALS patient decision making and to derive a decision-making model for specialised ALS multidisciplinary care.

Methods: Fifty-four respondents (32 health professionals, 14 patients and 8 carers) from two specialised ALS multidisciplinary clinics participated in semi-structured interviews between April 2011 and May 2012. Interview topics were derived from the patient decision-making literature body, refined in reference to ALS. Audio recordings of interviews were transcribed, coded and analysed thematically.

Results: Comparison of health professional, patient and carer perspectives revealed broad agreement on decision making for ALS symptom management and quality of life. Six factor domains were reported to influence patient-centred decision making. These were the process of decision making; use of a patient-centred focus; timing and planning; information sources; engagement with specialised ALS services; and access to non-specialised services. In addition, psychosocial factors and continually changing symptoms, including physical, cognitive and behavioural deterioration, impacted on patients' capacity to participate. Participants agreed that specialised ALS multidisciplinary clinics offered an optimal setting for decision making. Nevertheless, issues of timing of evidence-based care delivery and the role of carers were contentious.

Stakeholders perceived ALS decision making as a collaborative, complex and cyclical process. The derived model is embedded in the decision-making environment of the specialised ALS multidisciplinary clinic. Health professionals, patients and carers form a decision-making triad, and move through a cycle of four interlinked stages. Patients move within and between each stage of the model until ready to proceed. The first stage, 'Patient Engagement', identifies the participants and establishes their values, preferences and expectations. In stage two, 'Option Information', information and guidelines on the available management options are determined, including the optimal timing for implementation of each choice. During the third stage, 'Deliberation', patients weigh up the risks and benefits, and decide between proceeding with an option, deferring their decision, or choosing to do nothing. The final 'Implementation' stage results once an option is chosen.

Discussion and conclusion: Participant engagement in ALS patient-centred decision making is tested by the dynamic nature of the disease, limited treatment options and patient and family distress. The roles and expectations of stakeholders influence the decision-making process. Respect for patient autonomy may conflict with delivery of well-timed, evidence-based care. This empirically derived model captures these complexities and offers a framework for health professionals, researchers and policy makers in this challenging environment.

C10   QUALITY OF LIFE, DEPRESSION AND PERCEIVED SOCIAL SUPPORT IN THE COURSE OF ALS

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Keywords: quality of life, depression, social support, progression

Background: ALS is associated with severe physical function loss and major burden. However, there is evidence that hedonic quality of life (QoL), which refers to transient feelings, such as life satisfaction and happiness is known to be good in a majority of ALS patients. Depression rate is often low. QoL and depression are usually not associated with loss of physical function in cross-sectional analysis.

Objectives: The aim of the study is to determine the dynamics of hedonic QoL and depression in association with perceived social support over time.

Methods: ALS patients (n = 93) were interviewed in a prospective longitudinal approach thrice in the course of one year. QoL was assessed with the anamnestic comparative self-assessment (ACSA, range −5 for as bad as possible and +5 as good as possible), subjective QoL was assessed with the schedule for the evaluation of subjective quality of life (SEIQoL, range 0 for as bad as possible and 100 as good as possible). Depression was measured with the ALS depression inventory (ADI-12, range: 12–48; > 28 indicates clinically relevant depression). Perceived social support was evaluated using the emotional scale of the social support scale (SOZU K 22, range: 22–110). Physical function of ALS patients was measured using the ALS functional rating scale revised form (ALS-FRS-R, range: 0–48).

Results: Indicators of good psychosocial adaptation such as high SEIQoL (score > 70 on a scale of 100 indicates a good QoL) and neutral to positive global QoL (ACSA), and low depression (ADSK) were stable throughout the study. Furthermore, perceived emotional social support was stable despite significant physical function decline.

Discussion: Subjective QoL was good and depression was low and stable throughout the study despite significant decrease in physical function. This is in line with the literature on stable and good subjective QoL in the course of ALS. Therefore, hedonic QoL is independent of physical function

in ALS and differences in QoL between patients are likely due to pre-existing individual differences (Roach et al., 2009). Good and stable emotional social support is a prerequisite for good QoL and low depression rate.

Conclusion: The stability of QoL is a highly important finding as an anticipated poor QoL was one of the most important reasons for requesting physician assisted suicide in Oregon (Ganzini et al., 2009) and probably in other locations as well.

C11   UNDERSTANDING QUALITY OF LIFE IN MOTOR NEURONE DISEASE: QUALITATIVE EXPLANATIONS FROM THE TRAJECTORIES OF OUTCOME IN NEUROLOGICAL CONDITIONS STUDY (TONiC)

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Background: Previous studies have reported that motor neurone disease (MND) may affect perceived quality of life (QoL). Quantitative relationships between QoL and other factors (eg physical deterioration and social support) have been reported, while qualitative researchers have examined conceptualisation of QoL. Although these studies provide insight, more work is needed on contributory factors and their relationships.

Objectives: The aim of this study was to identify factors that affect perceived QoL and to understand how they function.

Methods: Semi-structured interviews were conducted with 19 MND patients (male = 13, bulbar onset = 8, mean age = 62.7 yrs). The mean duration since the diagnosis was 22.4 months (range: 1–110 months). The interviews were audio recorded and transcribed verbatim. Thematic analysis was employed in which inductive coding systems were developed to identify themes.

Results: The analysis identified four influential factors for QoL: Social Factors, Spirituality, Personal Qualities and Coping Strategies. Social Factors entailed four subthemes which were having significant others, getting understanding from others, receiving support, engaging with social activities, and making contributions to others. Positive outcomes from these were empowerment, enjoyment and existential meaning for life, while negative outcomes were psychological distress and behavioural withdrawal. The data also showed that some individuals are more psychologically independent than some others to whom empowerment from external support was vital for perceived QoL. Spirituality was found to have positive influence in giving individuals peace and purpose of life. Personal Qualities included positive attitude, locus of control and self-efficacy. While presence or absence of positive attitude was found to be consistent throughout an interview, degree of self-efficacy was observed to shift depending on situations as well as illness severity. Similarly, both internal and external locus of control were occasionally displayed by the same individual. Coping Strategies consisted of both emotion-focused coping and problem-focused coping. Among the four main themes, Social Factors, Spirituality and Personal Qualities appeared to be more influential than Coping Strategies to QoL. Coping strategies were perceived to be rather influenced by other three factors. In addition, Social Factors and Spirituality were found to influence Personal Qualities, yet this varied between patients.

Discussion and conclusion: QoL of individuals with MND was explored using a qualitative approach. The analysis showed that Social Factors, Spirituality, Personal Qualities, and Coping Strategies are influential to QoL. The impact of the Social Factors and Personal Qualities in particular may be stressed for their influence on QoL. Individual differences observed suggest the importance of the patient-centred approach. Specifically, study suggests the importance to recognise those who may benefit from receiving support and to understand the shifts in their sense of locus of control and self-efficacy over time.

SESSION 3A RNA PROCESSING AND DYSREGULATION

C12   HNRNP A3 BINDS TO GGGGCC REPEATS OF PATIENTS WITH C9ORF72 MUTATIONS: CONSEQUENCES FOR RAN TRANSLATION

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Keywords: C9orf72, RAN translation, hnRNPA3

Genetic analysis revealed the hexanucleotide repeat expansion GGGGCC within the regulatory region of the gene C9orf72 as the most common cause of familial amyotrophic lateral sclerosis and the second most common cause of frontotemporal lobar degeneration. Since repeat expansions might cause RNA toxicity via sequestration of RNA binding proteins, we searched for proteins capable to bind to GGGGCC repeats. *In vitro* transcribed biotinylated RNA containing hexanucleotide GGGGCC or, as control, AAAACC repeats were incubated with nuclear protein extracts. Using stringent filtering protocols 20 RNA binding proteins with a variety of different functions in RNA metabolism, translation and transport were identified. A subset of these proteins was further investigated by immunohistochemistry in human autopsy brains. This revealed that hnRNP A3 formed neuronal cytoplasmic and intranuclear inclusions in the hippocampus of patients with C9orf72 repeat extensions. Confocal microcopy showed that these inclusions belong to the group of the so-far enigmatic p62 positive/TDP-43 negative inclusions characteristically seen in autopsy cases of diseased C9orf72 repeat expansion carriers. Thus we have identified one protein component of these pathognomonic inclusions. We are now investigating if hnRNPA3 affects transport or translation of the GGGGCC-containing repeat RNA. Moreover, we will present data on the potential of primary cells from patients with C9orf72 mutations as simple models for the disease pathology. Repeat associated non-ATG repeat translation can occur not only from sense-strand repeat RNA transcripts but also from antisense transcript. We have therefore raised antibodies against the potential reverse strand specific di-peptide repeat (DPR) proteins poly-AP and poly-PR. Both antibodies specifically recognize a portion of characteristic p62-positive inclusions in the hippocampi and cerebella of C9orf72 repeat expansion carriers. Thus multiple DPR proteins are deposited in brains of patients with C9orf72 repeat extensions. Finally, we will present data on the potential of primary cells from patients with C9orf72 mutations as models for the disease pathology. We have investigated iPS cells from human patients with C9orf72 repeat extensions. Cells were so far grown for 80 days after differentiation into neurons. We searched for deposition of DPR proteins translated directly from the C9orf72 repeat extensions as well as for p62 pathology. However, so far no depositions were found. Cells growing for longer time points are currently under investigation.

C13   THE ROLE OF RNA BINDING PROTEIN HNRNP K IN ALS AND FTD

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Keywords: TDP-43, FUS, hnRNP K

Background: Tar DNA binding protein 43 kDa (TDP-43) has been identified as the major pathological protein of ALS and FTLD-U. In pathological brain and spinal cord tissue, one of the hallmarks of the diseases is the relocalisation of TDP-43 from the nucleus to the cytoplasm, where it undergoes various post-translational modifications. Alterations in neuronal RNA processing are characteristics of many neurodegenerative disease states. Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a RNA-binding protein that is implicated in apoptosis, neurodegeneration and is upregulated in various cancers. Preliminary results suggest that TDP-43 and hnRNP K are closely related. The clinical and pathological commonality between ALS and FTD suggests these diseases share underlying mechanisms that constitute to the diseases. This is reinforced by TDP-43 positive inclusions being a signature feature in both ALS and FTLD-U cases.

Objective: The objective of the present study is to decipher the molecular mechanism of TDP-43 cytoplasmic protein accumulation and to elucidate why greater toxicity and proteinopathies are associated with mutated TDP-43 in ALS and FTD patients.

Methods: NSC-34 (mouse hybridoma line as a motor neuron-like model) cells stably transfected to express either normal (wild-type, WT) or mutant (A315T or Q331K) TDP-43 labeled with the fluorescent cherry tag, human fibroblasts derived from an ALS patient harboring an M337V mutation in TDP-43 and control human fibroblasts with normal TDP-43 were employed. For knock down experiments, SH-SY5Y cells were transiently transfected with hnRNP K siRNA at 10nM using DharmaFECT reagent.

Results: In response to stress induced by sodium arsenite, NSC-34 cells expressing WT TDP-43 showed robust phosphorylation of cytosolic, but not nuclear hnRNP K, while this was not evident in the mutant cell lines. Under the same conditions, in the cytosol of cells containing the Q331K mutation, there was almost a complete loss of expression of RNA binding proteins FUS and hnRNP K with lesser changes to expression induced by A315T or WT TDP-43. Fibroblasts taken from a patient with ALS displayed dramatically reduced expression of hnRNP K compared to control fibroblasts. In parallel studies, we found that knockdown of hnRNP K by siRNA in SH-SY5Y cells attenuated stress granule (SG) formation and TDP-43 accumulation.

Discussion and conclusion: The data demonstrate that disease-causing mutations in TDP-43 are capable of inhibiting a key stress-associated change to cytosolic hnRNP K and FUS. Due to loss of hnRNP K and FUS in cells expressing mutant TDP-43, the data may suggest that WT TDP-43 can regulate the stability of hnRNP K and other RNA binding proteins, while the mutations inhibit this. This reinforces a key role for altered hnRNP K processing in TDP-43-mediated disease. Finally, this research provides further support for an important and complex interaction between TDP-43 and hnRNP K.

C14 TDP-43'S NEUROTOXICITY IS MEDIATED BY FRAGILE X PROTEIN AND SPECIFIC MRNA TARGETS

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Keywords: TDP-43, RNA dysregulation, FMRP

Background: Recent findings have demonstrated that defects in RNA processing are at the heart of pathophysiological mechanisms leading to neuronal dysfunction and death (1). Several RNA-binding proteins have been implicated in ALS both at the level of pathology and as causative agents (2). Of these, TDP-43 is a member of the hnRNP A/B family and harbors two RRM domains, an NLS, an NES and a prion-like C terminus domain. TDP-43 is localized to the nucleus under physiological conditions but associates with cytoplasmic RNA granules during stress (3).

Objectives: We used a Drosophila model of ALS (4, 5) to test the hypothesis that by associating with cytoplasmic RNA granules, TDP-43 leads to the sequestration of specific RNA binding proteins and subsequent dysregulation of their mRNA targets, which in turn may provide the basis for neural toxicity.

Methods and results: In a genetic screen for candidate RNA-binding proteins that modulate TDP-43 phenotypes, we have identified Fragile X Mental Retardation protein (FMRP), which has an established role in RNA transport and translation. FMRP and TDP-43 form a complex in mammalian cells and bind *in vitro*. Genetic interactions in Drosophila show that loss of FMRP enhances TDP-43 toxicity in neurons. In contrast, FMRP overexpression alleviates TDP-43 phenotypes both in the retina and motor neurons. To further evaluate the significance of FMRP as an effector of TDP-43 toxicity, we tested whether *profilin* and *futsch*, two established FMRP targets, can also modulate TDP-43 phenotypes. Interestingly, mutations in either *profilin* or *futsch* can alleviate TDP-43 neurodegeneration in the retina but only *futsch* rescues locomotor defects caused by TDP-43 overexpression.

Discussion and conclusion: These findings support our model of TDP-43-induced RNA dysregulation and point to specific mRNA targets regulated by FMRP as effectors of TDP-43 toxicity *in vivo*, in a tissue specific manner.

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C15 MISSENSE MUTATIONS IN DIFFERENT DOMAINS OF THE MOUSE TDP43 GENE CAUSE DIVERSE EFFECTS ON RNA METABOLISM

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Keywords: TDP43, RNA, model

Background: TDP43 is a ubiquitously expressed prevalently nuclear protein involved in RNA splicing, RNA stability and miRNA processing. Post-mortem analysis of patients with amyotrophic lateral sclerosis (ALS) has shown that TDP43 is depleted from the nucleus and accumulates in cytoplasmic neuronal inclusions, which are the pathological hallmark of the disease. Mutations in TDP43 have been found to be causative of a proportion of ALS familial cases reinforcing the primary importance of this molecule in the disease pathogenesis.

The pathogenic mechanism by which TDP43 acts is unclear, and both loss of nuclear function (LOF) and gain of function (GOF) mechanisms have been proposed.

TPD43 null mice have not proved a helpful tool to study TDP43 LOF due to early embryonic lethality, and transgenic mice overexpressing both wild type and mutant TDP43 have been reported to develop to neurotoxicity making the mechanistic dissection of mutant toxicity challenging.

Objectives: Here we characterize two novel mouse TDP43 mutant lines, carrying ENU-induced point mutations in the mouse endogenous *Tardbp* gene in order to study the effects of TDP43 mutations expressed at physiological levels in the mammalian central nervous system.

Results: The two mutations lay in two different domains of the TDP43 protein: (a) the F210I mutation is located in the RRM2 domain, involved in RNA binding, and indeed strongly reduces the RNA-binding capacity of the protein; and (b) the M323K mutation is in the glycine-rich C-terminal domain where the majority of pathogenic mutations are found. Both mutations are very disruptive and in homozygosity cause either late foetal or early post-natal lethality. We analyse TDP43 splicing target transcripts to show that the two mutations mainly have opposing effects. We perform RNAseq on embryo brains of homozygous, heterozygous and control animals from both lines and find that the two mutations have very different effects on RNA expression and splicing.

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Conclusions: Our results underline the importance of studying models with physiological expression levels of TDP43 mutations and shed light on the different effects on RNA metabolism caused by the TDP43 loss and gain of function.

C16   SYSTEMIC DYSREGULATION OF TDP-43-BINDING MICRORNAS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: microRNA, TDP-43, epigenetic

Background: Amyotrophic lateral sclerosis (ALS) is a classical neurodegenerative disease affecting primarily motor neurons. Central aspects of this disease are pathological aggregates of the TARDBP-coded protein TDP-43 in the vast majority of ALS patients. TDP-43 binds to and is involved in processing of both coding RNAs and a small subset of microRNAs (miRNAs), which are key epigenetic regulators of transcriptome plasticity and suspected to play a role in the pathogenesis of neurological diseases.

Methods: We therefore hypothesized that nine recently identified TDP-43-binding miRNAs might be dysregulated in ALS patients and quantified their levels in cerebrospinal fluid (CSF), serum and immortalized lymphoblast cell lines derived from ALS patients and healthy controls.

Results: We found that five of the TDP-43-binding miRNAs were dysregulated in the CSF and six in the serum of sporadic ALS cases (in at least 22 per experimental group). Differentially altered miRNAs together with a poor correlation between CSF and serum levels indicate a systemic dysregulation of miRNA biogenesis or degradation also outside the CNS, in line with the ubiquitous expression of TDP-43. The most downregulated miRNA-132 could be confirmed in lymphoblast cell lines from sporadic, TDP-43, FUS and C9orf72, but not SOD1 mutant patients. This parallels the TDP-43 pathology found in most ALS cases, but usually not in SOD1 mutant ALS patients.

Conclusion: Taken together, we report a systemic and genotype-dependent dysregulation of TDP-43-binding miRNAs in human biomaterial that might reflect an easily accessible biological measure of TDP-43 dysfunction in ALS.

C17   STRESS GRANULE (SG) DYNAMICS IS REGULATED BY AUTOPHAGIC MACHINERY IN FUS-RELATED ALS

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Keywords: FUS, stress granules, RNA-binding protein

FUS is a DNA-/RNA-binding protein found to be mutated in some cases of both sporadic and familial forms of ALS. It is still not clear how ALS-causing mutations in FUS leads to motor neuron degeneration. Here, we exploited a Drosophila model and mammalian neuronal cell lines to elucidate the role of the RNA-binding ability of FUS in regulating FUS-mediated toxicity. To determine the role of the RNA-binding ability of FUS in ALS, we mutated FUS RNA-binding sites (F305, F341, F359, and F368) to leucines and generated RNA-binding-incompetent mutants (4F-L) with and without ALS causing mutations R518K or R521C. We found that mutating 4F to L residues makes FUS RNA-binding-incompetent. We observed that ectopic expression of RNA-binding-incompetent FUS in fly brain, eyes, and motor neurons strongly blocks neurodegenerative phenotypes as compared to RNA-binding-competent FUS carrying ALS causing mutations. Interestingly, RNA-binding deficient FUS strongly localized to the nucleus of Drosophila motor neurons and mammalian neuronal cells, whereas FUS carrying ALS linked mutations was distributed to the nucleus and cytoplasm.

Importantly, we found that incorporation of mutant FUS into stress granules (SG) is dependent on the RNA-binding ability of FUS. SGs are dynamic aggregates composed of proteins and RNA that are formed when cells are under a variety of stresses. We observed that normally cytoplasmic SGs rapidly disassemble when stress conditions end, whereas cytoplasmic SGs formed in ALS patient cells having a FUS mutation fail to disassemble. This suggests that mutant FUS sequesters proteins and RNAs important for cellular homeostasis and the defect in disassembly of cytoplasmic SGs contributes to ALS. Interestingly, we found that induction of autophagy by rapamycine was sufficient to accelerate disassembly of cytoplasmic stress granules in ALS-patient cells. Furthermore, we observed that ectopic expression of atg1 strongly suppressed mutant FUS-related neurodegenerative phenotypes in our fly model of ALS. We strongly believe that these findings suggest potential therapeutic targets for ALS.

SESSION 3B COGNITIVE AND PSYCHOLOGICAL CHANGE

C18   SCREENING FOR COGNITIVE AND BEHAVIOUR CHANGE IN ALS

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Keywords: cognition, behaviour, screen

Background: Despite the increase in awareness of ALS as a multisystem disorder, the cognitive status of the majority of ALS patients attending clinics remains unknown. Standard assessments for the detection of dementia are of limited use due to the range of physical problems in ALS with difficulties speaking, drawing and writing. Other cognitive screening tools fall short of a comprehensive assessment, measuring a single cognitive domain (executive functions) which is therefore not sensitive to the heterogeneity of cognitive change in ALS.

Objectives: This study presents the new Edinburgh Cognitive and Behaviour ALS Screen (ECAS), specifically developed for ALS patients with either limb or speech physical disability for use by health care professionals within the clinic. This multi-domain screen consists of a 15- to 20-minute interview and separate carer behaviour scale and is designed to detect the specific profile of cognition and behaviour changes in ALS and to differentiate it from other disorders.

Methods: Forty-eight ALS patients (none with evident dementia), 40 healthy controls and 20 carers were recruited. The ECAS, includes an ALS-Specific score (executive functions including social cognition; fluency; and language); an ALS Non-specific score (memory and visuospatial functions); a carer behaviour screen of five domains characteristic of frontotemporal dementia (FTD).

Results: Data from healthy controls produced abnormality cut-offs of 77/100 ALS-Specific score; 24/36 ALS non-specific score; 105/136 ECAS Total. 29% of patients showed abnormal ALS-Specific Scores, and 6% also showed abnormal ALS Non-specific scores. The most prevalent deficit occurred in language functions (35%) followed by executive functions and fluency (23% each). 40% of carers reported behaviour change in at least one domain, while 15% met criteria for possible FTD. The most prevalent symptoms were apathy, loss of sympathy/empathy and change in eating behaviour.

Discussion and conclusion: The ECAS is an effective brief assessment for ALS which determines the presence, severity and type of cognitive and/or behavioural changes. This is an essential first step to managing such symptoms and will enable streamlining of care into appropriate pathways and tailoring intervention.

C19   HIGH RATES OF COGNITIVE AND BEHAVIORAL IMPAIRMENT IN A LARGE PROSPECTIVE ALS STUDY

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Keywords: FTD, cognitive, behavior

Background: Patients with ALS develop frontotemporal dementia (FTD) and more subtle cognitive impairment (ALSci) and behavioral impairment (ALSbi) (1). To recognize this extramotoneuronal involvement is essential to understand the pathogenesis of ALS. The development of screening tests to identify cognitive impairment is an important advancement in the field, to provide clinicians with effective tools for use in busy clinics.

Objectives: To investigate the cognitive and behavioral functioning of patients prospectively enrolled in the longitudinal ALS COSMOS study, and to identify the relationship between cognitive and behavioral symptoms and clinical variables among patients.

Methods: We have completed enrollment of 364 patients with probable or definite ALS based on El Escorial criteria, 258 of whom had completed baseline data for inclusion in this study. Patients were administered three well-recognized screening cognitive-behavioral measures: the ALS-Cognitive Behavioral Screen (ALS-CBS) (2). Frontal Behavioral Inventory-ALS version, and the Abrahams Written Fluency Test (3). Cognitive and behavioral impairment was defined based on screening tests and not a full exam, using the ALS-CBS.

Results: The sample was 42.9% female, with a mean age of 60.9 (SD = 9.9), mean education of 15.0 years (SD = 3.0), mean FVC of 79.7 (SD = 22.1) and mean ALSFRS-R of 36.3 (SD = 6.3). 9.8% of the sample met criteria for FTD with cognitive impairment and 18.4% of the sample met criteria for FTD with behavioral impairment. An additional 64.4% and 13.4% met ALSci and ALSbi criteria, respectively. While verbal fluency was not correlated with FVC or bulbar involvement, impaired performance on concentration and mental tracking subtests was associated with reduced FVC and reduced bulbar functioning. Increased behavioral impairment was associated with reductions in FVC, bulbar functioning, and ALSFRS-R and increased PBA symptoms.

Discussion: These diagnostic cut-offs were established using the ALS-CBS screening exam and are not to be confused with a formal neuropsychological diagnosis. Concentration and mental tracking were associated with FVC and bulbar impairment, with verbal fluency being unassociated with these clinical traits.

Conclusions: Approximately 10–15% of patients met FTD criteria, with larger proportions of patients meeting criteria for ALSci and ALSbi. Behavioral impairment correlated with more bulbar dysfunction, more PBA, and lower socioeconomic status. Illness duration did not correlate with ALSbi or ALSci, suggesting that extramotor involvement may be independent of the primary motor disease process.

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C20   NEUROPSYCHIATRIC SYMPTOMS APPEAR VERY EARLY IN ALS – AND DO NOT AFFECT SURVIVAL

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Keywords: neuropsychiatric symptoms; behavioural changes; survival

Background: While the presence of cognitive deficits has been well-established in ALS, the identification and assessment of neuropsychiatric symptoms is less developed to date. Apathy has been recognised but presence of other challenging behaviours such as disinhibition and stereotypical behaviour, common to FTD, is less understood. Finally, concomitant ALS-FTD is associated with worse prognosis but there is little understanding if milder degrees of dysfunctional behaviour also affect survival in ALS.

Objectives: (1) To investigate patient susceptibility to neuropsychiatric symptoms and classic motor symptoms in ALS; and (2) To examine the impact of neuropsychiatric symptoms on survival.

Methods: Two hundred and ninty-nine patients (Limb onset = 159 and bulbar onset = 60) were included, following current diagnostic criteria for ALS. Behavioural symptoms were measured via a short version of the Cambridge Behavioural Inventory (CBI-R) and classic ALS symptoms via the ALSFRS-R.

For the analysis of symptom susceptibility (behavioural vs classic motor), a Rasch analysis was employed (n = 219). For the survival analysis, Cox proportional hazard regression models were applied (n = 159 patients with complete information), which included classic motor symptoms and neuropsychiatric symptoms.

Results: The Rasch analysis demonstrated that behavioural symptoms appear earlier than the development of classic

motor symptoms in ALS. The differences in behavioural scores did not affect survival; patients with abnormal scores in neuropsychiatric domains did not have a different rate of survival than those without (Chi square: 3.447, $p = 0.328$, -2 log likelihood 377.341).

Discussion: The notion of neuropsychiatric symptoms appearing prior to classic motor symptoms in ALS is novel and potentially controversial, given that it brings ALS even closer to FTD, while confirming that ALS is a multisystem disorder, affecting different areas concomitantly. The marked and early presence of the neuropsychiatric symptoms are likely to affect clinical decisions (eg, PEG insertion; compliance in use of respiratory devices, etc.). The fact that neuropsychiatric symptoms alone do not affect survival confirms their pervasiveness, and only the co-occurrence of ALS and FTD seems to affect prognosis.

Conclusions: Neuropsychiatric symptoms are an early feature in ALS, but have not been thoroughly evaluated to date. They appear earlier than classic motor symptoms. Importantly, these behavioural symptoms alone do not seem to affect survival in ALS, which in turn confirms their pervasive nature in ALS.

C21   BEHIND THE CURTAIN OF DYSARTHRIA: THE NATURE OF LANGUAGE IMPAIRMENT IN MND

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Keywords: language, speech, cognition

Background: While aphasic-type impairments have been documented in MND since the early 1990s (1), the common clinical view is still that communication difficulties can be attributed solely to dysarthria. Yet recent evidence raises the possibility of central processing impairments. Naming deficits have been frequently reported, and comprehension of grammatical contrasts has also been shown to be impaired in some patients (2). However, there is also increasing evidence of spelling errors, which could suggest contribution of a more phonologically based impairment. Japanese authors have reported MND patients with selective errors writing phonologically based kana characters, but not semantically based kanji (3), while others have reported substitution and transposition of letters in patient writing (4).

Objectives: To examine the nature of speech and language deficits in people with MND and the extent to which: (1) expressive communication impairment cannot be solely explained by dysarthria; (2) receptive language deficits impact upon communication; and (3) clinical management should be adapted to meet the communication needs of patients.

Methods: Twenty-five MND patients from across Scotland with changes in speech and/or language were tested using a battery of experimental and standardised tests of naming, spelling, grammatical comprehension, prosody and phonological and orthographical awareness. Patients were screened for levels of dysarthria and hearing impairment and results were compared to those of 25 age-, sex- and education-matched controls.

Results: Of the 25 MND patients, 13 (52%) performed two or more SD below the control mean on at least one linguistic assessment. As a group, MND participants performed significantly worse than controls on measures of naming (p = 0.001), spelling (p = 0.006), grammatical comprehension (p = 0.001), emotional, but not acoustic prosody (p = 0.002) and orthographical awareness (p = 0.003). However, the pattern of impairment was not global, with five patients showing dissociation of performance on naming and grammatical comprehension and six between naming and spelling.

Discussion and conclusion: Communication impairment in MND cannot solely be attributed to dysarthria in every patient. Dissociation in performance for some patients between linguistic measures suggests that there may be multiple impairment profiles, and that naming assessment alone cannot give an accurate measure of linguistic abilities. This study highlights the importance of multidimensional assessment of language for clinical management, particularly with regard to AAC strategies.

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C22   EFFICACY OF HYPNOSIS-BASED TREATMENT IN ALS AND ITS EFFECT ON THE CAREGIVER: RESULTS OF A SIX-MONTH LONGITUDINAL STUDY

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Keywords: psychological intervention, hypnosis, quality of life

Background: Amyotrophic lateral sclerosis (ALS) has a catastrophic psychological impact on affected patients and their caregivers. Previous research has shown that psychological welfare and quality of life (QoL) are crucial factors for the patient's prognosis. However, although would be strongly needed, there is a lack of research on the efficacy of psychological intervention and by now there are no clinical intervention guidelines.

Objectives: Following the promising results of a pilot research (1), the aim of the study is to investigate the long term effects of an hypnosis-based intervention on psychological health and perception of secondary physical symptoms in patients and the indirect effect on their caregivers.

Methods: Fifteen typical ALS patients, (8 females and 7 males) and their closest caregiver (10 females and 5 males) participated in the study. The patients were treated with a domiciliar hypnosis intervention and self-hypnosis training protocol lasting 1 month conducted by a trained clinician. Anxiety and depression levels were measured with the Hospital Anxiety and Depression Score in patients and caregivers before treatment (T0) and in three follow-up phases: immediately following the treatment (T1) and after 3 (T2) and 6 months (T3). QoL and perceived physical symptoms changes were investigated in patients, in every study phase, with ALS Assessment Five Items Questionnaire and the ALS Specific Quality of Life-Revised questionnaire. Test–retest analyses were conducted with Wilcoxon signed-rank tests and the Cliff's delta was used to estimate effect sizes.

Results: One month pre-post treatment improvement in depression, anxiety, and physical and affective facets of QoL were found significant. Decreases in perception of physical symptoms such as cramps, sleep disorders, emotional lability, and fasciculations were reported by our patients. Reduced anxiety and improvements in QoL were maintained at the follow-ups at 3 and 6 months. Significant improvements in caregiver's anxiety and depression values were measured at T2 and maintained at T3.

Discussion: The collected data clearly showed a positive effect of the hypnosis-based intervention on the patients and indirectly on their caregivers. The preservation and improvement in some aspects of anxiety, depression and QoL after 6 months appears as the most relevant clinical result of our study.

Conclusions: To the best of our knowledge, this is the first report on psychological intervention protocol effectiveness on ALS patients. The findings provide initial support for using hypnosis to manage ALS physical consequences and mainly to cope its dramatic psychological implications for patients. Hypnosis could represent an eligible treatment where classical psychological colloquy may result impossible in severe bulbar symptomatology or locked-in condition.

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C23   DIGNITY THERAPY: A PSYCHOTHERAPEUTIC INTERVENTION TO ENHANCE THE END OF LIFE EXPERIENCE FOR PEOPLE WITH MOTOR NEURONE DISEASE AND THEIR FAMILY CARERS

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Keywords: psychosocial support, dignity therapy, quality of life

Background: Quality of life for people with MND is primarily dependent on spiritual, existential, relationship and support factors. There have been numerous calls for psychosocial interventions which address hope, meaning and existential distress, though psychotherapies addressing these needs are

rare. Studies have also documented the substantial burden and distress experienced by MND family carers, as well as the absence of targeted interventions to alleviate this distress.

Dignity Therapy is a brief psychotherapy designed for people with terminal diagnoses to enhance a sense of purpose, meaning, and overall quality of life. Dignity Therapy has proven successful at relieving psychosocial distress in patients, as well as providing comfort to family members, though most patients in previous studies had cancer diagnoses.

Objectives: This study examined whether Dignity Therapy enhances the end of life experience for people with MND and their family carers. Feasibility and acceptability were assessed, and any required modifications to the therapy or other special considerations were examined.

Method: Twenty-six people with MND and 17 family carers from Western Australia participated in the study, which adopted a pre-post design to investigate the impact of Dignity Therapy on both people with MND and their family carers. Secondary outcome measures were also obtained 1 week after the therapy using feedback questionnaires completed by patients and family carers.

Results: Dignity Therapy was beneficial to people with MND, especially in the areas of encouraging acceptance (70%), strengthening identity (70%), helping to address unfinished business (65%) and helping to feel they could still play an important role (65%). Acceptability was high, with 84% reporting the therapy was helpful to them and 92% reporting the therapy was satisfactory. Acceptability was also high with family carers, with 94% finding it helpful to their family member and 75% recommending it to others. Seventy-five percent of family carers believed the dignity therapy document would be a comfort during bereavement. Low base rates of distress precluded being able to demonstrate significant post-intervention differences on measures of distress. These results mirror those found in previous studies of dignity therapy with cancer patients and their carers.

Mild cognitive decline and pseudo-bulbar affect were found to have minimal impact on the acceptability and feasibility of the psychotherapy. Dysarthria (speech impairment) did not impact acceptability or feasibility and the therapy was successfully completed using assisted communication devices.

Discussion and conclusion: Dignity Therapy is acceptable to people with MND and their family carers, who report numerous benefits from the therapy. This psychotherapy can be successfully delivered using assisted communication devices.

SESSION 4A *IN VITRO* MODELLING

C24 SMALL-MOLECULE SCREENING FOR NEUROPROTECTIVE AGENTS

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Effective therapies for motor neuron disease are desperately needed. Unfortunately, clinical trials of compounds that showed some efficacy in preclinical models have thus far largely failed. Although the precise reasons for these failures are debated, the need for preclinical models that better predict the results of clinical trials seems clear. Moreover, the majority of patients with motor neuron disease have no identifiable genetic cause. How to develop preclinical models that faithfully represent patients with sporadic motor neuron disease has been unclear. To help address these unmet needs, we developed primary neuron models of motor neuron disease that exhibit several disease-relevant phenotypes. Of particular interest are motor neuron disease models developed from human motor neurons and astrocytes differentiated from patient-derived induced pluripotent stem cells (iPSC) reprogrammed from fibroblasts of patients who have genetic and sporadic motor neuron disease. To develop human iPSC models of motor neuron disease, we applied high-throughput automated single-cell longitudinal analysis, which uncovered several disease-relevant phenotypes. In turn, we are using these primary murine and human neuron models of motor neuron disease to screen for genetic and pharmacological modifiers that mitigate disease phenotypes and show therapeutic potential and to help medicinal chemistry efforts to optimize leads from primary screens.

C25 RNA-INDUCED TOXICITY FROM THE C9ORF72 ALS/FTD REPEAT EXPANSION IS MITIGATED BY ANTISENSE INTERVENTION

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Background: A hexanucleotide 'GGGGCC' repeat expansion in the noncoding region of the *C9ORF72* gene has recently been identified in ~30% of familial and up to 10% of sporadic ALS cases (1–3) and is therefore the most common genetic abnormality associated with ALS to date. Since the function of the C9ORF72 protein is unknown and a C9ORF72 rodent model has not yet been generated, few methodologies exist to begin to elucidate the pathogenicity behind this repeat expansion. However, repeat expansions in non-protein coding regions are the known cause of other neuromuscular disorders (eg DM1/2) and pathogenesis is, in part, due to the accumulation of *cis* acting expanded repeat-containing RNA that sequester *trans* acting RNA binding proteins (RBP).

Objectives: To generate and characterize iPS neurons and astrocytes from C9ORF72 ALS patient fibroblast, compare with human autopsied CNS tissue, and develop antisense therapeutics (ASO) that target toxic RNAs to rescue aberrant iPS phenotypes.

Methods: Using high-throughput screenings, we have profiled the transcriptome of C9ORF72 patient-derived iPS motor neurons and astrocytes as well as human autopsy tissue. Proteome arrays and biochemical assays were employed to identify GGGGCC RNA-binding partners. RNA FISH-IF was utilized to validate the presence and co-localization of toxic RNA foci and (GGGGCC) exp RNA-binding partners *in vitro* and *in vivo*. RAN translation products were detected with monospecific antibodies. Glutamate toxicity studies were performed using propidium iodide imaging and LDH assays.

Results: We have identified RNA-induced toxicity through accumulation of intranuclear (GGGGCC) exp RNA, an aberrant transcriptome, RBP sequestration, and dose dependent susceptibility to excitotoxicity in patient-derived C9ORF72 ALS iPS neurons. RAN translation products were present in iPS neurons – but did not contribute to toxicity. Importantly, ASO treatment rescues the C9ORF72 phenotype.

Discussion and conclusion: Using iPS-differentiated neurons, we have 1) identified intranuclear (GGGGCC)exp RNA foci, 2) described dysregulated gene expression in C9ORF72 ALS tissue that match iPS cell lines, 3) have identified (GGGGCC)exp RNA binding partners, and 4) determined that C9ORF72 iPS neurons are highly susceptible to excitotoxicity. Importantly, all of these pathogenic characteristics are mitigated with antisense therapeutics to the C9ORF72

transcript or repeat expansion despite the presence of repeat associated non-ATG translation (RAN) products. Taken together, these data indicate a toxic RNA gain-of-function mechanism as a cause of C9ORF72 ALS. These studies also provide candidate antisense therapeutics as well as human pharmacodynamic biomarkers for drug actions.

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C26   COMPARISON OF DISEASE MECHANISMS AND THERAPEUTIC INTERVENTIONS IN PRIMARY CULTURE MODELS OF MULTIPLE FAMILIAL FORMS OF ALS/MND

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Background: Culture and animal models of familial ALS due to mutations in SOD1 (ALS1) have been used extensively for preclinical studies of candidate therapies for ALS. The discovery of additional genes linked to ALS provides the opportunity to compare disease mechanisms and therapeutic efficacy in multiple models. Our laboratory has established primary culture models of ALS1, ALS6 (*FUS* mutations) and ALS10 (*TARDP* mutations). A common factor is increased propensity of ALS-causing mutant proteins to aggregate and form inclusions in motor neurons; however, the pathways disrupted and factors contributing to cell-type vulnerability could vary, conferring differential sensitivity to intervention. This study is comparing how mutants of SOD1 and of the RNA-binding proteins, FUS and TDP43, exert toxicity in motor neurons.

Objectives: (1) Previous studies established a role for protein misfolding, glutamate receptor activation, calcium dysregulation, mitochondrial dysfunction and ER stress in the toxicity of mutant SOD1. The aim is to determine if these factors apply to mutant FUS and TDP43. (2) Upregulation of protein chaperones protects motor neurons from mutant SOD1. The aim is to determine if increasing expression of heat shock proteins prevents FUS and/or TDP43 from mislocalizing and aggregating in the cytoplasm, as well as downstream consequences in motor neurons.

Methods: Dissociated cultures of murine spinal cord-DRG are matured for 3–6 weeks. Human WT or ALS-causing mutants are expressed in motor neurons by intranuclear microinjection of expression plasmids along with a fluorescent dextran marker. Cytosolic Ca^{2+} is measured using fura-2. Mitochondrial morphology is visualized by co-expression of mitochondrially targeted eGFP/dsRed. Intracellular localization

is assessed by indirect immunocytochemistry or by incorporating an eGFP tag in the sequence.

Results: (1) In contrast to $SOD1^{G93A}$, neither FUS nor TDP43 mutants increased cytosolic Ca^{2+}. Mitochondrial length was decreased in motor neurons expressing mutant FUS, but not to the severe extent as $SOD1^{G93A}$. Although the calcium channel inhibitor, lomerizine, the AMPA glutamate receptor antagonist, CNQX, and riluzole-inhibited mutant SOD1 toxicity, results to date indicate that they do not affect cytoplasmic accumulation or formation of inclusions of mutant FUS or TDP43. (2) Upregulation of HSPs prevents multiple aspects of mutant SOD1 toxicity, despite only partially limiting Ca^{2+} dysregulation. Experiments with FUS and TDP43 mutants are ongoing.

Discussion and conclusion: Many factors could contribute to the failure of clinical trials of compounds showing efficacy in models of ALS1, one being that ALS is a syndrome with multiple causes. This study points to early contribution of calcium dysregulation in toxicity of mutant SOD1, but not FUS or TDP43. The identification of multiple genes and pathways linked to familial ALS provides an opportunity for enhanced preclinical testing and stratification of patients in trials.

C27   TARGETING RNA FOCI SHOWS A THERAPEUTIC EFFECT IN iPSC-DERIVED MOTOR NEURONS FROM C9ORF72 REPEAT PATIENTS

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Keywords: iPSC, antisense

Background: Expansions of a hexanucleotide repeat (GGGGCC) in the noncoding region of the C9ORF72 gene were recently found to be the most common cause of familial ALS (C9-ALS) and also frontotemporal lobar degeneration, and are also present in other neurological diseases. While the mechanism of repeat toxicity remains unclear, accumulations of RNA GGGGCC rich foci are a hallmark of pathological changes seen in these conditions.

Objectives: To report a cellular model of C9-ALS using patient-derived induced pluripotent stem cells (iPSCs), and use it as a tool to understand the mechanism of the disease.

Results: The absolute number of motor neurons generated from C9-ALS and control lines was similar, suggesting no overt neurodegeneration occurred. Interestingly, the expansion showed somatic instability in patient-derived neuron cultures, and transcription of the repeat was paradoxically enhanced, leading to accumulations of GGGGCC repeat containing RNA foci selectively in C9-ALS patient cells. Repeat containing RNA foci co-localized with hnRNPA1

suggesting they are functional structures that can alter splicing and transcription to influence disease pathogenesis. Accordingly, C9-ALS patient motor neurons showed a distinct transcriptional profile with changes in several genes including DPP6, previously implicated in ALS genome wide association studies. Finally, we show that antisense oligonucleotides targeting the C9ORF72 transcript can suppress RNA foci, and reverse gene expression alterations in patient cells.

Discussion and conclusion: These data support the concept of a toxic RNA gain of function mechanism in C9-ALS patients. Furthermore, using patient-specific motor neurons as a model, we suggest that ASOs are a promising therapeutic strategy for C9ORF72 expansion diseases.

C28   THERAPY DEVELOPMENT FOR ALS/MND AND FRONTOTEMPORAL DEMENTIA WITH C9ORF72 EXPANSION: ANTISENSE OLIGONUCLEOTIDE MEDIATED REDUCTION IN NUCLEAR RNA FOCI

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Keywords: C9orf72, RNA foci, antisense oligonucleotides

Background: Expanded hexanucleotide GGGGCC repeats in a non-coding region of the *C9orf72* gene were recently identified as the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD). The pathogenic mechanisms of this expansion are not understood but initial observations point to either a loss of function of the endogenous *C9orf72* gene, or a toxic gain of function of the expanded RNA, mediated either by sequestration of RNA-binding proteins or by production of aberrant polypeptide(s) through repeat-associated non-ATG-dependent (RAN) translation.

Objectives: Our objectives were to determine the contribution of loss of function versus toxic gain of function in neurodegeneration linked to C9orf72 expansion and to test the efficacy and tolerability of therapeutic strategies lowering *C9orf72* RNA.

Methods: We used locked nucleic acid probes to identify expanded RNA foci by *in situ* hybridization in peripheral cells and autopsies from C9-ALS/FTD patients. We determined the sensitivity of foci to RNAse H-dependent antisense oligonucleotides (ASOs) versus cytoplasmically acting siRNAs, and we used genomic approaches to define RNA profiles linked to *C9orf72* expansion. Tolerability to *C9orf72* depletion in the adult nervous system was determined by behavioral testing of normal mice after intraventricular delivery of a mouse *C9orf72*-specific ASO.

Results: We identified accumulation of expanded RNAs into nuclear foci in multiple cell types including spinal motor neurons, cortical neurons, Purkinje cells and glial cells from C9-ALS/FTD patients. Such foci are not seen in nervous systems from sporadic ALS, Parkinson's disease, MAPT mutation or non-neurologic controls.

The presence of RNA foci in patients supports a toxic gain of function that may be tackled by therapeutic strategies lowering the production of abnormal RNAs. We demonstrated the efficiency of nuclear RNase H-dependent ASOs to target *C9orf72* RNA transcripts and reduce the formation of foci. Importantly, isoform-specific ASOs lowering only RNAs that contain the expansion reduced foci without altering overall *C9orf72* transcript levels. By comparison, cytoplasmically acting siRNAs against *C9orf72* reduced overall RNA levels, but did not reduce foci.

Finally, RNA alterations, relative to unaffected controls or sporadic ALS samples, were identified in fibroblasts and spinal cords from C9-ALS/FTD patients by genome wide RNA analysis approaches. Depletion of *C9orf72* in control fibroblasts and in spinal cords from normal adult mice resulted in gene expression changes that do not significantly overlap with the *C9orf72* RNA signature, thereby providing evidence that *C9orf72* RNA alterations are not due to C9orf72 loss of function. We determined that reducing endogenous *C9orf72* for several months in the central nervous system of normal mice was well-tolerated, a crucial step towards the development of ASO therapy in C9-ALS/FTD.

Conclusion: A toxic gain of function is likely fundamental in C9-ALS/FTD pathogenesis. Strong evidence supports that ASOs targeting *C9orf72* will be a powerful therapeutic strategy.

SESSION 4B QUALITY OF CARE

C29 ADVANCE CARE PLANNING IN ALS – THE ROLE OF THE PHYSICIAN

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The importance of advance care planning (ACP) in ALS has been increasingly recognized in recent years. ACP is much more than completing advance directives and nominating a health care proxy, important as though these elements are. ACP is an ongoing process that starts when the diagnosis is communicated and continues throughout the course of the illness.

As ALS is an illness with a relatively predictable course in most patients, prevention is the key to successful disease management and palliative care. Several triggers have been identified by expert consensus (1) for starting end of life discussions in ALS:

- The patient or family asks – or 'opens the door' – for end-of-life information and/or interventions;
- Severe psychological and/or social or spiritual distress or suffering;
- Pain requiring high dosages of analgesic medications;
- Dysphagia requiring feeding tube;
- Dyspnea or symptoms of hypoventilation, a forced vital capacity of 50% or less; and
- Loss of function in two body regions.

Importantly, end-of-life decisions in ALS need regular revisiting, as ALS patients have been shown to change their priorities and wishes for the end-of-life, sometimes dramatically, within short periods of time.

The role of the physician in this process is manifold:

- To be aware of the legal regulations governing ACP and end-of-life decisions, which vary significantly between countries.
- To be attentive to clues indicating the patients desire to discuss ACP.
- To actively inquire for the presence of the trigger points described above.
- To ensure inclusion of the relatives in ACP, with the patient's consent.
- To alert the patient early enough of impending treatment decisions, so as to allow time for reflection and discussions within the family.
- To bring the patient in contact with other patients who had to face similar decisions (eg PEG) in order to facilitate the decision process.
- To respect the patients' decisions even when they appear irrational and/or self-harming.

At its core, ACP is an ongoing communication process which rests in particular on the active listening skills on the physician's side. As Søren Kierkegaard said:

"If we want to help somebody, we must first find out where he stands. This is the secret of all caring. Those who cannot do this, are stuck with an illusion if they think they can help others. In order to really be able to help somebody, I must understand more than he does - but first and foremost I must understand what he understands." (2).

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C30 THE ISSUES OF END-OF-LIFE CARE PLANNING – THE FINAL STAGES

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In the care of people with ALS/MND, there is a need to prepare for end-of-life care early in the disease progression. This is particularly the case if there are communication difficulties, due to bulbar disease, or evidence of cognitive change. It can be argued that preparation for end-of-life care starts at diagnosis and certainly how the diagnosis is given and the information provided at this time can greatly influence the attitudes of the patient and family and their concerns as the disease progresses.

Advance care planning is widely advocated across the world, although the exact procedures will vary from country to country. However, the overall aims will be to allow discussion of the patient's wishes while they can clearly express them, so that all involved, patient, family and health and social care professionals, can act appropriately at the end of life, when the patient is unable to express their wishes. However, there are often barriers to these discussions:

- From the person with ALS/ MND;
  - Fear of disease progression
  - Magical thinking – "if I talk about dying I will die"
  - Concerns for the family/carers
- From the family;
  - Fear of the unknown;
  - Not wanting to upset the patient; and
  - Fearing that discussion of dying will reduce hope and affect the disease progression.
- From the professionals;
  - Patients will become upset
  - Families do not want these discussions;
  - Concerns about giving up hope;
  - It is too hard to talk about these issues.

However, there is the need to face these issues and help patients and families express their wishes, and hopes, and facilitating them in achieving their wishes if possible, such as visiting friends or family or significant places. If the wishes are not known this can lead to conflict and increased issues for all involved in the care of the patient. These conflicts may include the disagreements within families, with varying views of the patient's wishes, and conflicts within teams as they all have different ideas about what the patient would have wished and fail to be objective as they may bring their own views into these discussions.

Thus careful discussion throughout the disease progression will allow the views and wishes of the patient, in collaboration with their family and professional carers, to be expressed and recorded. In this way the patient's care can be co-ordinated effectively and then all can be supported – patient, family and professionals.

SESSION 5A THERAPEUTIC STRATEGIES

C31 TARGETING IMMUNE RESPONSES IN NEURODEGENERATIVE DISEASE

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Keywords: monocytes, brain diseases, innate immunity

The concept of the central nervous system (CNS) as an immune privileged organ has led to a common misunderstanding that it is not an active immunological organ, guarded from its surroundings by the blood-brain barrier (BBB). Recent advances in this field clearly demonstrate that the CNS is a highly immunologically active organ, with complex immune responses mostly based on innate immune processes. Such responses implicate a continuum of heterogenous cell types both inside the CNS, in the periphery and at their interface, the BBB. We recently found that the progressive cognitive decline and decrease in expression of numerous synaptic markers and neurotrophins in the brain of mouse models of Alzheimer's disease (AD) correlated with major changes in the proportions of peripheral blood monocyte subsets when compared with age-matched controls. Indeed, there is a defect in the production of circulating M1 monocytes in APP/PS1 mice, whereas the population of M2 monocytes remains normal in this mouse model of AD. In this regard, low levels of macrophage colony-stimulating factor (M-CSF) were recently measured in patients with presymptomatic AD or mild cognitive impairment (MCI), which together with low levels of other haematopoietic cytokines predicted the rapid evolution of the disease towards a dementia diagnosis 2–6 years later. *In vitro*, exposure of mouse microglia to M-CSF enables the acidification of their lysosomes and, subsequently, the degradation of internalized Aβ. Injecting M-CSF to transgenic mice that spontaneously develop AD on a weekly basis prior to the appearance of learning and memory deficits prevented cognitive loss. The treatment also restored the population of M1 monocytes in the circulation and greatly decreased Aβ levels. In addition, M-CSF treatment resulted in the stabilization of the cognitive decline state in transgenic mice that already had Aβ pathology. These results are quite encouraging as they suggest that stimulating circulating M1 cells may have a great therapeutic potential for AD. This could explain why recent clinical trials in AD have revealed that anti-inflammatory drugs not only failed to improve cognitive functions, but worsened disease in some patients. It is therefore likely that stimulating monocytic cells may be a new therapeutic avenue for treating brain diseases, such as AD. In this presentation, will show new data regarding the potent effects of new molecules to stimulate innate immune cells as a preventive and curative treatment for brain diseases. We will also show the central role of the neurovascular unit in diseases of the CNS and how it can be targeted for novel therapeutic strategies.

C32 ACTIVATION OF THE BRAIN'S CHOROID PLEXUS FOR LEUKOCYTE TRAFFICKING AS A THERAPEUTIC APPROACH FOR ALS

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Keywords: vaccination, immunomodulation, choroid plexus

Background: Circulating $CD4^+$ T cells were shown to play a beneficial role in controlling the local neuroinflammation in Amyotrophic lateral sclerosis (ALS); in the mutant superoxide dismutase 1 G93A (mSOD1) murine model of ALS, T-cell deficiency contributes to increased neuronal loss, while boosting T cell levels reduces it (1).

Objectives: We recently identified the brain's choroid plexus (CP) as a compartment in which CNS-specific $CD4^+$ T cells reside (2), and as a trafficking gate for "healing" leukocytes to the damaged CNS (3). Based on these findings, we hypothesized that the activation of this compartment for leukocytes trafficking is dysregulated in ALS, resulting in poor immune cell recruitment to the CNS.

Methods: Adult C57BL/6J wild-type (WT) and mSOD1 mice were immunized with MOG_{35-55} or ovalbumin peptide. Cerebrospinal fluid, CP and spinal cords were collected and analyzed by flow cytometry, qPCR, immunohistochemistry and Luminex systems. CP epithelial cells were cultured *in vitro*.

Results: We found that activation of the CP for leukocyte trafficking is dependent on IFN-g production by T cells residing in the CP stroma. In mSOD1 mice, starting from the asymptomatic stage, the CP showed lower numbers of IFN-g-producing $CD4^+$ T cells, lack of activation for leukocyte trafficking, and poor recruitment of $CD4^+$ T cells to the cerebrospinal fluid and the spinal cord. Active immunization of mSOD1 mice with a CNS-derived peptide, at the asymptomatic stage of the disease, activated their CP to facilitate leukocyte trafficking, and led to the specific accumulation of $Foxp3^+$ regulatory T cells in the spinal cord gray matter, adjacent to the motor neurons – reaching more than 40% of the $CD4^+$ T cell population 28 days post immunization. Importantly, immunized mSOD1 mice showed increased average survival of 15 days (from 144.2 ± 3.8 to 159.5 ± 2.2 days); at a stage at which 90% of the vaccinated mSOD1 mice were still alive, only 10% of the untreated mice survived.

Discussion and conclusion: Taken together, we propose that activation of the CP for leukocyte trafficking is beneficial in ALS, yet do not spontaneously occur along disease course. It is therefore possible that immunization with a self-peptide, which initiates an immune cascade leading to recruitment of inflammation-resolving immune cells to disease sites, may lead to the development of hitherto unexplored therapeutic vaccines for fighting ALS, and perhaps other neurodegenerative diseases.

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C33   RECOMBINANT HUMAN-DERIVED MONOCLONAL ANTIBODIES TARGETING MISFOLDED SOD1 AS NOVEL THERAPEUTICS FOR THE TREATMENT OF ALS

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Keywords: SOD1, human antibodies, immunotherapy

Background: Misfolded superoxide dismutase 1 (SOD1) accumulates in both familial and sporadic ALS, suggesting a generalized role as a drug target for the treatment of ALS. Conformational neo-epitopes within misfolded SOD1 can trigger immune responses in human subjects leading to B-cell memory for misfolded SOD1.

Objectives: To identify, clone and recombinantly express human monoclonal antibodies selectively targeting misfolded SOD1 with high affinity, and to evaluate their pharmacological efficacy in transgenic mouse models of ALS.

Methods: Immune repertoires from cohorts of healthy elderly human donors without clinical signs of neurodegenerative disease were screened for memory B cells against misfolded SOD1. High-affinity and selective antibodies were cloned, recombinantly expressed in CHO cells and characterized both *in vitro* and on post-mortem spinal cord sections from ALS patients. Pharmacological efficacy *in vivo* was tested in two independent transgenic mouse lines overexpressing human mutant SOD1.

Results: We cloned, expressed and characterized high-affinity human antibodies that selectively target misfolded SOD1 while not binding to physiological SOD1 dimers. In spinal cord tissue sections obtained at autopsy from patients with ALS or human control subjects, the lead antibody selectively bound to misfolded SOD1 in motor neurons of ALS patients. In transgenic mice, chronic antibody treatment significantly reduced SOD1 pathology and rescued spinal cord motor neurons resulting in significantly reduced muscle atrophy and better motor functions. The lead antibody increased survival times by up to 2 months, was efficacious in two independent SOD1 transgenic mouse lines, and was effective through both peripheral and intracerebroventricular administration routes.

Discussion and conclusion: High-affinity, recombinant human-derived antibodies against conformational epitopes in misfolded SOD1 selectively bind pathological SOD1 deposits in spinal cord sections obtained from ALS patients while sparing physiological SOD1 dimers in human control subjects. At therapeutic doses, these antibodies are effective in independent transgenic mouse models of ALS. The lead antibody is a promising candidate for the development of a safe and efficacious immunotherapy for ALS.

C34   NANOBODY AGAINST SOD1 REDUCES IN VITRO AGGREGATION, RESCUES SOD1-INDUCED AXONOPATHY AND EXTENDS SURVIVAL IN ALS MODELS

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Keywords: ALS, SOD1, nanobody

Background: Many neurodegenerative diseases such as Alzheimer's and Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS) are caused by a mutant protein which, through the presence of this mutation, gains a function that is toxic for the cell. Reducing the levels of the pathogenic mutant protein is an obvious strategy to treat patients suffering from these disorders. ALS is an adult-onset fatal neurodegenerative disease that is familial in about 10% of patients. In about 20% of patients with familial ALS (FALS) the disease is caused by gain-of-toxic-function mutations in the gene encoding Cu/Zn-superoxide dismutase (SOD1). Reducing the levels of the pathogenic SOD1 is an interesting strategy to treat patients with mutant SOD1-associated familial ALS, and maybe also of patients with sporadic ALS.

It has been described that camelids and some sharks produce functional antibodies devoid of light chains, where the single N-terminal domain was fully capable of antigen binding. This so-called VHH domain can be cloned and the product, called nanobody, shows high-target specificity and affinity, and low inherent toxicity. Furthermore nanobodies are highly soluble, extremely stable, have refolding capacity, can recognize hidden antigenic sites, can be administered by means other than injection and are easy to manufacture. Nanobodies may have applications as diagnostic markers and as therapeutics in many fields of medicine. Furthermore, nanobodies have very limited immunogenicity due to high sequence similarities with human VH family III. Finally, and particularly important for their potential use in neurodegenerative disorders, some nanobodies have been shown to cross the blood-brain barrier efficiently.

Objectives: To reduce the toxic effect of mutant SOD1 in the motor neurons, we have raised anti-SOD1 heavy chain antibodies in dromedaries and alpacas, and cloned its N-terminal antigen binding VHH region, coding for an anti-SOD1 nanobody (SOD1 nanobody). We have investigated the therapeutic potential of this nanobody, by studying its effect on mutant SOD1-induced toxicity *in vitro* and *in vivo*.

Results and discussion: We have used two different approaches: transfection or injection of constructs that express the SOD1 nanobody and also the administration of the SOD1 nanobody itself. *In vitro*, we have demonstrated that SOD1 nanobody has high affinity for SOD1 and blocks its fibril formation. It also prevents mutant SOD1 aggregation when

different cell lines are transfected with mutant SOD1. We have found this effect when the cells are co-transfected with mutant SOD1 and the SOD1 nanobody and when the cells are treated with the SOD1 nanobody. *In vivo*, its injection and its administration rescues mutant SOD1-induced axonopathy in a zebrafish model for ALS. It also delays onset and extends lifespan of transgenic mice that overexpress SOD1[G93A], murine model for ALS, when they are treated regulary with the SOD1 nanobody intracerebroventriculary. This positive effect is also significant once the neurodegenerative symptoms have started. We have also demonstrated that it binds to SOD1 in human ALS spinal cord. Our data demonstrate the potential use of the SOD1 nanobody as a possible therapy for ALS.

C35   AAV9-MEDIATED SOD1 DOWNREGULATION AS A FUTURE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: gene therapy, non-human primates, motor neurons

Background: *In vivo* transgenic removal of mutant SOD1 from motor neurons as well as glial cells can significantly delay the disease progression and improve survival in ALS mouse models. Moreover, misfolding of SOD1 in sporadic ALS patients has been reported, thus opening the potential of targeting SOD1 for all ALS patients. Therapeutic strategies to reduce SOD1, including antisense oligonucleotides and viral-delivered RNA interference (RNAi) have been attempted with varying success, and to date, only antisense oligonucleotides have been tested clinically.

Objectives: Here we investigate the feasibility and efficacy of post-natal downregulation of SOD1 using a novel approach of AAV9-mediated shRNA delivery in the SOD1G93A mice. The ultimate goal of this study is to determine the safety of this approach and to define guidelines for a clinical trial by testing its efficacy in non-human primates (NHP).

Methods: SOD1G93A mice, overexpressing human mutant SOD1, were intravenously injected at P1, P21 or P85 with 3×10^{12} viral particles of scAAV9-SOD1 shRNA or scAAV9-GFP virus. 3 NHP were injected intrathecally at the sacral level with 1×10^{13} vg/Kg scAAV9-GFP-shSOD1 to determine biodistribution and SOD1 suppression level.

Results: P1 injected mice showed widespread spinal cord transduction of scAAV9-SOD1 shRNA virus targeting both neuronal and non-neuronal cells, while P21- and P85-injected mice showed predominant astrocytic transduction. Importantly, the transduced cells persisted throughout the life span of the treated animals and reduced levels of mutant SOD1 protein were detected. P1- and P21-treated mice showed improved performance on behavioral tasks. P1-injected mice showed significant delay in the disease onset, whereas P21- and P85-injected mice showed delay in disease progression. Importantly, all three treatments significantly improved the median survival of SOD1G93A mice between 30 and 50 days. Excitingly, intrathecal administration of scAAV9-SOD1 shRNA in NHP also resulted in efficient spinal cord transduction and significant SOD1 reduction. We also determined the safety of scAAV9-SOD1 shRNA administration by injecting wild-type mice at P1 or P21 and monitoring them up to 6 months of age. Injected wild-type mice showed no difference in their motor performance compared to controls. Hematology, serum profiles and histopathology studies showed no significant alterations.

Conclusions: Taken together, we report here one of the longest survival extensions in two regularly used als mouse models using scaav9 sod1 shrna. Thus, the success of post-natal suppression of SOD1 toxicity by AAV9-mediated shrna delivery in ALS mouse models provides a sound basis for its clinical translation. Also, the efficient knockdown of SOD1 in non-human primates and long-term safety assessment in wild-type mice further strengthen the suitability of this approach, thus setting the stage for human clinical trials.

SESSION 5B EPIDEMIOLOGY

C36　ENDEMIC ALS: IS THERE ANYTHING WE CAN LEARN FROM CLUSTERS?

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Keywords: genetics, epidemiology, risk factors

Clusters are groupings of health-related events having temporal and/or geographical correlation. Spatial and temporal clusters in amyotrophic lateral sclerosis (ALS) have been reported, implying a genetic predisposition of the affected population and/or an environmental influence. A classical example of clustering in ALS is represented by the Western Pacific form (the ALS-Parkinson-Dementia-Complex, PDC) observed in the island of Guam, the Kii Peninsula of Japan, and in Western New Guinea. In these areas, the incidence of the disease has been found, in the fifties, about 50- to 100-fold higher than elsewhere in the world. Since then, the decreased incidence and the changed phenotype suggested that the disease could be caused by an environmental factor. Beta-N-methyamino-L-alanine (BMAA), a cyanobacterial-derived neurotoxin found in cycad flour and shown in the brains of ALS patients, has been implicated as a possible cause. At the end of the ALS-PDC outbreak, several other environmental factors have been postulated to trigger ALS, mostly in the context of occupational exposures. These include pesticides, metals, solvents, electromagnetic fields, head trauma, physical exercise, and smoking. However, none of these factors has been unequivocally found to increase the risk of ALS to a great extent. In recent years, a higher than expected risk of ALS was reported among veterans of the First Gulf War. This risk was apparently limited to the decade following the war, which again suggests an environmental exposure. These findings are consistent with other reports and support a possible correlation between ALS and military service. Exposure to toxic agents, traumatic brain injury (TBI), manual work, and strenuous physical activity have been all implicated. Another cohort recently found at higher risk of ALS is represented by professional soccer players. This cohort has some exposures in common with the veterans (TBI, physical exercise) and, interestingly, is likewise characterized by a frequent onset of the disease under the age of 45 years. This latter finding can be explained by a possible interaction between genetic and environmental factors.

Despite being useful instruments for the investigation of risk factors for ALS, at present studies on the temporal or geographical variation of the frequency of the disease have failed to provide evidence of a strong causal relationship between ALS and several environmental factors. Even BMAA has not been definitely found to represent a significant risk factor, as cyanobacteria are ubiquitous and increasingly prevalent, and good animal models are still lacking. Nevertheless, in light of the recent advances in the genetics of ALS, the study of clusters should continue and the role of environmental factors should be assessed in combination with the investigation of the genetic background of the affected individuals.

C37　FEASIBILITY ASSESSMENT OF AN EPIDEMIOLOGIC STUDY OF ELECTROCONVULSIVE THERAPY AND MOTOR NEURON DISEASE

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Keywords: magnetic fields, electric shocks, electroconvulsive therapy

Background: Electrical environments, mostly in occupational settings, have been linked to development of neurodegenerative diseases including motor neuron disease (MND). In occupational epidemiologic studies, the association for MND appears to be stronger with occupational titles designating electrical occupations than with estimated levels of magnetic field (MF) exposure. It has been suggested that exposure to electric shocks (ES) in these occupations rather than the MF exposure may be the causal factor in these associations. It is difficult to separate occupational exposure scenarios for ES and MF as they frequently occur together. Electroconvulsive therapy (ECT), a relatively commonly used treatment for some psychiatric conditions, presents a scenario where ES occurs without significant exposure to MF.

Objectives: To evaluate the feasibility of a large-scale international epidemiologic study of ECT and MND.

Methods: We sought information on treated person rate (TPR) for ECT and frequency of ECT utilization among psychiatric patients in several counties. We also estimated MND incidence and estimated the anticipated number of MND patients among patients receiving ECT.

Results: In Western countries, the TPR tends to range between roughly 2 and 5 per 10,000 residents per year. It has been estimated that ECT might be used in about 5% of hospitalizations for psychiatric diseases in Denmark; the same proportion is estimated at 2% in Sweden. It was also estimated that between 1987 and 2011, approximately 190 patients with depression or schizophrenia died from MND in Sweden. Under the assumption of no association, five of these patients would be expected to have received ECT. In the US Veterans Health Administration, among almost 190,000 patients with major depression, 307 (0.16%) received ECT in a 5-year period.

Discussion and conclusion: ECT represents a unique exposure scenario where the potential effect of ES on MND risk may be evaluated uncoupled from the potential effects of MF exposure. To further assess the feasibility of an epidemiologic study of this kind, the availability of data on ECT will be required in several countries, along with the availability of additional medical records information to evaluate if early symptoms of MND may potentially confound the association. Due to rarity of both the exposure (ECT) and the disease (MND), an international collaboration will be required to pool data from several countries. An epidemiologic study investigating the ECT–MND association will contribute to our understanding whether the consistently reported association between electrical occupations may be potentially explained by the etiologic role of ES in MND development.

RIGHTS LINK

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C38   N-3 AND N-6 POLYUNSATURATED FATTY ACID INTAKE AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS: POOLED RESULTS FROM FIVE COHORT STUDIES

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Keywords: diet, polyunsaturated fatty acids, epidemiology

Objective: Prior research has suggested the possible role of oxidative stress and inflammation in the pathogenesis of amyotrophic lateral sclerosis (ALS). Dietary n-3 and n-6 polyunsaturated fatty acids (PUFA) may affect oxidative stress and inflammation and could thus contribute to determine ALS risk. Therefore, using a prospective design, we examined whether the fatty acid composition of the diet is related to ALS risk.

Methods: Risk of ALS associated with total dietary fat and fat subtypes as well as individual PUFAs was investigated in five prospective cohorts: the Cancer Prevention Study II Nutrition Cohort (CPS-II), the Multiethnic Cohort (MEC), the National Institutes of Health-Association of American Retired Persons Diet and Health Study (NIH-AARP), the Health Professionals Follow-up Study (HPFS), and the Nurses Health Study (NHS). Diet was assessed via food frequency questionnaires. Incident cases of ALS (NHS and HPFS) and ALS deaths (all cohorts) were identified by biennial follow-up questionnaires or from the National Death Index and confirmed by review of the medical records or death certificates. A total of 994 ALS cases occurred among 995,755 participants (476,980 women and 522,775 men) during a median follow-up of 12.0 years. We applied Cox proportional hazards regression to calculate cohort-specific multivariable-adjusted risk ratios (RR) across quintiles of intake of the dietary variables, and pooled the cohort-specific results using random-effects methods.

Results: In analyses adjusted for age, gender, smoking, and body mass index, a greater n-3 PUFA intake was associated with a reduced risk of ALS (RR for the highest versus the lowest quintile: 0.63; 95% CI: 0.50–0.79; P trend < 0.001). An inverse association was observed for both α-linolenic acid (ALA; 18:3 n-3) and long-chain marine n-3 fatty acids – for ALA, the RR for a 1 g/day increment (corresponding to about 1 tablespoon of canola oil per day) was 0.65 (95% CI: 0.48–0.88; P = 0.005), and for marine n-3 the RR for a 200 mg/day increment (about two servings of fatty fish per week) was 0.86 (95% CI: 0.75–0.99; P = 0.03). Total fat and other types of dietary fats, including n-6 PUFA, were not associated with ALS risk.

Discussion: The results of this large longitudinal study suggest that consumption of foods high in n-3 PUFAs may reduce ALS risk.

C39   INTERACTION BETWEEN HFE POLYMORPHISMS AND CUMULATIVE LEAD EXPOSURE ON THE RISK OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oxidative stress, iron metabolism, hemochromatosis

Background: We previously found an association between cumulative lead (Pb) exposure and risk of amyotrophic lateral sclerosis (ALS). Polymorphisms in the hemochromatosis (HFE) gene-involved in regulating iron uptake-have previously been associated with ALS. Oxidative stress may contribute to ALS pathophysiology, and excess iron can amplify toxic effects of Pb related to oxidative stress. No study has examined the possible modifying role of HFE on the association of cumulative Pb exposure with ALS risk.

Objectives: To determine whether HFE polymorphisms alter the association of cumulative Pb exposure with ALS risk.

Methods: We measured bone Pb – a biomarker of cumulative Pb exposure-using K-shell-X-Ray Fluorescence in 100 neurologist-confirmed ALS cases and 192 controls from New England. We genotyped H63D and C282Y HFE polymorphisms; participants were considered variant carriers or not for each. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for ALS using logistic regression adjusted for age, sex, education, and smoking. To assess effect modification by HFE polymorphism, we included in one model terms for Pb (continuous), H63D and C282Y genotypes (separately), and separate cross-product terms between Pb and the polymorphisms.

Results: We found an elevated OR for ALS among H63D variant carriers (OR: 5.30; 95% CI: 1.81–15.52), but not C282Y carriers, as seen in other studies. The cross-products terms indicated that the OR for association of patella lead with ALS among C282Y variant carriers was 15 times larger (15.48; 95% CI: 1.54–155.9) than that among those without C282Y variants, while the OR among H63D variant carriers was about half as large (0.43; 95% CI: 0.20–0.93) as that among those without H63D variants.

Discussion and conclusion: Our results suggest that the H63D and C282Y HFE genotypes modify the association of Pb with ALS risk, but in opposite directions. Thus effects on iron are unlikely to explain the findings since both H63D and C282Y increase labile iron.

C40 ALS MULTICENTER COHORT STUDY OF OXIDATIVE STRESS (ALS COSMOS): THE STUDY METHODOLOGY, RECRUITMENT, AND BASELINE DEMOGRAPHICS AND DISEASE CHARACTERISTICS

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Keywords: ALS disease progression, epidemiology, oxidative stress

Background: Although the majority of patients die within 3–4 years, the rates of disease progression and survival time are highly variable in ALS.

Objective: In a multicenter study design of newly diagnosed ALS patients without a family history, we prospectively investigated whether oxidative stress (OS) is associated with disease progression.

Methods: All de-identified patients seen at each study site were evaluated for study eligibility and enrollment. For enrolled patients, detailed clinical assessments and cognitive screening tests for frontotemporal dementia were performed. Extensive structured telephone interviews ascertained clinical, environmental, lifestyle, dietary, and psychological risk factors. Data collection was performed at baseline and at 3- to 6-month intervals using modified questionnaires for 30 months or until death or study drop out. We developed a biorepository including urine, DNA, plasma and skin biopsies for initial analyses of OS and lipid biomarkers and genetic screening of C9ORF72.

Results: A total of 356 patients were enrolled during a 36-month-enrollment period by 16 sites. The most common reasons that eligible patients were not enrolled into the study were (1) the study was not discussed, (2) patients refused to participate, and (3) patients were overwhelmed by the ALS diagnosis. Differences in insurance carriers/coverage were noted between study-eligible/non-enrolled and enrolled patients. The latter were more likely to have private insurance and less likely to be on Medicare ($P = 0.004$). Otherwise, there were no differences in the demographic and diagnostic features between these two groups. The enrolled patients were arbitrarily further grouped into the single high enrollment site ($n = 139$), median enrollment sites (> 0.6 patients/month; $n = 160$), and lower enrollment sites (< 0.5 patients/month; $n = 57$) to investigate differences in demographics and disease characteristics based on enrollment. Among these three groups, the high enrollment site and low enrollment sites had more neurologist-referred patients ($p = 0.007$). For diagnostic categories, the high enrollment site had more possible ALS and PMA diagnoses, but fewer definite ALS diagnoses compared to other sites ($P < 0.001$). The remaining demographic and disease characteristics did not differ by enrollment group.

Discussion: Although some minor differences were found between study-eligible/non-enrolled and enrolled patients and among different study sites, our study population is likely to represent the patient population seen at all study sites.

Conclusion: To our knowledge, this is the first prospective, interdisciplinary, in-depth multicenter epidemiological investigation of OS related to ALS progression. At the time of the presentation, we will show the results of the analysis of the baseline questionnaires and OS/lipid biomarkers. We believe data generated from our study will expand the understanding of the mechanisms involved in ALS prognosis (Grant support by NIEHS, R01ES016348, and MDA).

SESSION 6A CELL METABOLISM AND STRESS

C41 A MITOCHONDRIAL ETIOLOGY OF METABOLIC AND DEGENERATIVE DISEASES

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For half a millennium Western medicine has focused on anatomy and for the past century on nuclear DNA (nDNA), Mendelian, genetics. While these concepts have permitted many biomedical advances, they have proven insufficient for understanding the common "complex" diseases. In addition to anatomy, life requires energy and about 90% of energy comes from the mitochondrion. The mitochondrial genome consists thousands of copies of the maternally inherited mitochondrial DNA (mtDNA) plus between 1000 and 2000 nDNA genes. The mtDNA has a very high mutation rate, but the most deleterious mutations are removed by an ovarian prefertilization selection system. Hence, functional mtDNA variants are constantly being introduced into the human population, and the sequential accumulation of these mutations has permitted people to adapt to different regional environments as they migrated out-of-Africa to populate Eurasia and the Americas. As human migrations progressed, adaptive mtDNA variants became enriched in local environments giving rise regional groups of related haplotypes, or haplogroups, but these same adaptive variants can be maladaptive in alternative environments. A tRNA^{Gln} nt A4336G variant arose in between 8500 and 14000 years in Europe and persists today in 0.4% of European mtDNAs, yet this variant accounts for 3% late-onset Alzheimer's Disease (AD), 5% Parkinson Disease (PD), and 7% AD+ PD. A ND1 T3397C missense mutation (M31V) has arisen on the 4336 lineage but also independently and in both cases was found in AD+ PD patients. A missense mutation in the codon adjacent to the T3397C mutation, ND1 T3394C (Y30H), increases the penetrance of Leber Hereditary Optic Atrophy (LHON) mutations on macrohaplogroup N mtDNAs, but is adaptive for high altitude in Tibetans on a macrohaplogroup M. Haplogroup H mtDNAs, when combined with a homozygous frame shift mutation in the nDNA ADP/ATP translocase isoform 1 gene, are associated with mild cardiomyopathy, but when the ANT1 mutation is paired with haplogroup U mtDNAs the heart disease is severe and life-threatening. The phenotypic effects of mtDNA haplogroups can be augmented by nDNA variation and by deleterious germline or somatic mtDNA mutations. Confirmation that mtDNA variation contributes to common disease risk has been obtained by introducing mtDNA mutations into the mouse using mouse female embryonic stem cells (mfESCs). Mice harboring a COI T6859C V421A missense mutation develop a myopathy and cardiomyopathy while mice harboring a ND6 G13997A P25A missense mutation develop a LHON-like optic neuropathy. Simply mixing two normal mtDNAs from 129 and NZB mice together is sufficient to cause marked neuropsychiatric symptoms including reduced activity, heightened excitability, and severe learning defects. Hence, the primary genetic factors for predisposition to complex diseases may more often be regional mitochondrial variants rather than global anatomical gene variants.

C42 MUTATED SOD1 CAUSES REGION SPECIFIC DIFFERENCES IN CA^{2+}CYT DEPENDENT PROPERTIES OF MITOCHONDRIA FROM CNS OF SOD-1G93A MICE AND CA^{2+} DYSHOMEOSTASIS IN FIBROBLASTS OF FALS PATIENTS

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Background: The reasons why in experimental ALS models mitochondrial dysfunction and impaired Ca^{2+} homeostasis occur are not clear. It is also not understood why specific neuronal populations are selectively vulnerable in ALS.

Objectives: The aim of our project is to study the interrelation between mitochondrial dysfunction and the cytosolic Ca^{2+} concentration (Ca$^{2+}_{cyt}$) in mitochondria isolated from different CNS regions as well as in fibroblasts from fALS patients with SOD1 mutations.

Methods: Mitochondria isolated from spinal cord (SCM), brain stem (SM), and cerebellum (CM) of SOD1^{G93A}-mice were respirometrically and fluorimetrically investigated at varied Ca$^{2+}_{cyt}$ in comparison to wild type. Mitochondrial function was also investigated in permeabilized and intact fibroblasts (FM) of fALS patients compared to controls.

Results: CNS mitochondria of SOD1^{G93A}-mice exhibited mitochondrial impairments (diminished state 3$_{glutamate/malate/pyruvate}$ but unchanged state 3$_{succinate}$, increased flux control of complex I, decreased Ca^{2+} uptake rates and Ca^{2+} uptake thresholds, increased ROS formation). Also the stability of respirometric properties against Ca^{2+} stress (addition of 10 μM Ca$^{2+}_{cyt}$) were significantly reduced compared to wild type. Largest and most significant changes were observed in SCM > SM > CM of 130 days old SOD1^{G93A} mice. Similar changes were detectable also at 90 days old mice, but in a lesser extent. Since the Ca$^{2+}_{cyt}$ dependent stimulation of state 3$_{glutamate/malate}$ was found to be a typical property of neuronal mitochondria the extent of this parameter depends on the relative content of neuronal mitochondria (Gellerich unpublished). Whereas no difference was observed between SOD1^{G93A} and wild-type CM the Ca$^{2+}_{cyt}$ stimulation was diminished by 23% in SOD1^{G93A} SCM.

Also in FM of fALS^{SOD1} patients functional impairments were detected. Whereas the state 3$_{glutamate/malate/pyruvate}$ was not significantly altered the flux control coefficients of complex I increased by + 26%, (p < 0.05) and the activity ratio of complex I/complex IV tended to be decreased by 35 %. Morphologically mitochondria appeared to be swollen with fragmented or missing christae. Stationary Ca$^{2+}_{cyt}$ concentrations were decreased in intact fibroblasts of fALS^{SOD1}

patients compared to control cells -37% at endogenous conditions and -14% in 2mM Ca^{2+} containing medium. Treatment of ALS fibroblasts with 5 µM CoQ and 300 µM Trolox normalized in the most cases the elevated flux control coefficient for complex I.

Discussion and conclusion: We have shown in SOD1[G93A] mice and in fibroblasts of fALS patients that mutated SOD1 causes impairments of OXPHOS *and* mitochondrial Ca^{2+} metabolismus with consequences for an impaired Ca^{2+}-homeostasis. Our data support the view that ALS has also a generalized component potentially allowing the use of cultivated fibroblasts from ALS patients as a biomarker and for therapeutic studies.

C43   MITOCHONDRIAL METABOLIC MARKERS IN ALS FIBROBLASTS

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Keywords: skin fibroblasts, mitochondria, oxidative stress

Objective: We performed metabolic screening by medium throughput assays on fibroblasts from a cohort of ALS patients without family history and healthy controls. Our goal was to identify metabolic signatures that correlate with disease and to determine if metabolic abnormalities are linked to the clinical phenotype, establishing proof of concept that such alterations could represent viable biomarkers.

Background and significance: A large body of evidence links energy metabolism abnormalities to both sporadic and familial forms of ALS. We predicted that genetic and environmental factors could affect energy metabolism and predispose individuals to develop ALS and modulate the disease course. Different metabolic backgrounds could also affect the response to therapeutics. Thus, the failure to find effective compounds to treat the metabolic component of ALS may in part be attributable to the lack of understanding of energy metabolism as a disease modifier.

Methods: We surveyed a series of randomly selected 50 primary skin fibroblast lines from more than 170 skin biopsies of well-characterized ALS patients in the prospective ALS COSMOS study and 50 gender- and age-matched healthy controls. All patients were diagnosed and followed at multicenter study sites and had precise clinical and electrophysiological records. ALS and control skin fibroblasts were collected by skin biopsy, expanded, and frozen in liquid nitrogen after the first passage. For all assays, fibroblasts were used from early (2–3) passages. Metabolic assays were performed on fibroblasts, in triplicates. Mitochondrial membrane potential in medium containing glucose, pyruvate, and glutamine was measured with the potentiometric dye TMRM in an optimized plate reader assay. Background fluorescence was subtracted after addition of the uncoupler FCCP. Mitochondrial mass was estimated on the same cells with the membrane potential-independent dye mitoTracker green. Total cellular content of GSH was measured in a 96-well

assay by the conversion of monochlorobimane (MCB). All results were normalized by cell protein content.

Results: We found a significant increase in the average membrane potential of mitochondria from ALS fibroblasts. In the same cells, we found a decrease in the average mitochondrial mass. Furthermore, ALS fibroblasts had a significantly higher average content of GSH. We are currently in the process of analyzing the data to determine if correlations between the bioenergetic, mitochondrial, and redox state parameters exist with specific clinical subgroups of patients.

Conclusions: Our results disclosed changes in bioenergetic parameters in ALS fibroblasts that will be further investigated to assess the underlying mechanisms. Slow ADP phosphorylation could result in a build up of membrane potential and a downregulation of mitochondrial biogenesis. These changes may underscore a global shift of metabolism in ALS. The ongoing correlation studies will determine if there are metabolic signatures that distinguish different clinical disease parameters. (Grant support: NIH R01ES-16348 and MDA)

C44   ALTERED GROWTH HORMONE/INSULIN BALANCE IN HSOD1G93A MICE: IMPLICATIONS FOR INSULIN RESISTANCE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: metabolism, growth hormone, insulin resistance

Background: A significant metabolic component underlies amyotrophic lateral sclerosis ALS pathogenesis; GH deficiency (1), insulin resistance (2), glucose intolerance (3), and mitochondrial dysfunction (4) are observed in ALS. While defective metabolic balance negatively impacts ALS survival, the mechanism by which this contributes to ALS pathogenesis is unknown. To investigate the causes and consequences of altered metabolic balance in ALS, we conducted metabolic assessment in hSOD1G93A mice throughout disease progression.

Objective: Assess the expression of endocrine factors involved in the regulation of metabolic homeostasis in hSOD1G93A mice.

Methods: Male wild-type and hSOD1G93A transgenic mice were studied at four stages of disease; pre-symptomatic, onset of symptoms, mid-stage of disease, and end-stage of disease. We assessed pulsatile growth hormone (GH) secretion using a tail-tip method, and an in-house GH ELISA. Properties associated with the pulsatile pattern of GH secretion were analyzed by deconvolution analysis (5). Insulin tolerance was assessed via insulin tolerance test. Circulating levels of insulin, muscle glycogen content, and levels of non-esterified free fatty acids (NEFAs) were determined using commercial assays. Expression of mitochondrial respiratory chain complexes was determined by western blot.

Results: We report a dynamic GH secretory profile during ALS disease progression; we observe a significant increase in GH secretion at the onset of disease symptoms, and a decrease in GH secretion by the end-stage of disease (n ≥ 8 wild-type, n ≥ 7 SOD1G93A). We observe insulin resistance in hSOD1G93A mice by the mid-stage of disease, a time after the significant elevation in GH secretion. This coincides with a decrease in the expression of circulating insulin, decreased muscle glycogen, and an increase in the expression of NEFAs, and mitochondrial complexes II and IV in skeletal muscle (n ≥ 4/group).

Discussion and conclusion: We report the first definitive account of dynamic GH secretion in a transgenic mouse model of ALS. The development of insulin resistance, and changes in expression of circulating insulin is reflective of altered GH/insulin balance, and suggests that hypersecretion of GH at the onset of disease symptoms in hSOD1G93A mice underlies the development of insulin resistance in ALS. Moreover, our results imply that GH-induced insulin resistance causes a reduction in mitochondrial function, resulting in a decreased ability for muscle to utilise glucose whilst also driving accumulation of fat into skeletal muscle. Thus, GH-induced insulin resistance may underlie mitochondrial dysfunction in ALS.

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C45 MECHANISMS OF ER-GOLGI TRANSPORT INHIBITION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ER-Golgi transport, COPII, microtubules

Background: Previously we showed that ALS-mutant forms of SOD1, TDP43 and FUS inhibit ER-Golgi transport in cellular models of ALS, triggering ER stress and apoptosis by the accumulation of secretory proteins within the ER. Functional ER–Golgi transport requires the co-ordinated action of multiple proteins including COPII, which forms the coat protein surrounding vesicles as they bud from the ER. ER–Golgi transport also requires the presence of stabilized microtubules over which the vesicles are carried.

Objectives: The objectives of this study were to (i) determine the molecular and cellular mechanisms by which SOD1, TDP-43 and FUS impair secretory protein transport between the ER and Golgi apparatus, and (ii) to determine if inhibition of ER-Golgi transport can be detected *in vivo*.

Methods: VSVG^ts045 is a widely used marker to quantify ER–Golgi transport. Neuro2a cells were co-transfected with SOD1-EGFP, EGFP-TDP43 or HA-FUS and VSVG^ts045-mCherry, and budding of COPII vesicles from the ER was examined using an *in vitro* assay. The presence of stabilised microtubules was also examined by immunocytochemistry using antibodies against acetylated microtubule. Primary cortical neurons and motor neurons from SOD1 transgenic mice and non-transgenic mice at E13.5 were isolated and transfected with VSVG^ts045-mCherry.

Results: ER-derived vesicles obtained from cells expressing either ALS mutant TDP43 or FUS were depleted of COPII, suggesting that COPII vesicle formation is dysfunctional in these cells. In contrast, in cells expressing mutant SOD1, COPII vesicle formation appeared normal, but a high proportion of cells with destabilised microtubules was detected compared to controls. Inhibition of ER–Golgi transport was detected in both primary E13.5 motor neurons and cortical neurons obtained from SOD1^G93A mice, thus validating inhibition of ER–Golgi transport *in vivo* very early in pathology.

Discussion and conclusion: These findings show that dysfunction of ER–Golgi transport is a pathogenic mechanism shared by SOD1, TDP-43 and FUS. However, transport inhibition occurs by different mechanisms in cells expressing mutant SOD1 compared to mutant TDP43 or FUS. Furthermore, inhibition of ER–Golgi transport was present in embryonic motor neurons in SOD1 mice, implying that it occurs very early in pathogenesis.

C46 MUTANT TDP-43 LEADS TO PATHOLOGICAL ACCUMULATION OF SMN AND ITS NUCLEAR COMPLEXES IN MOTOR NEURONS

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Keywords: TDP-43, SMN, FTLD-U

Background: Abnormal copy number of *SMN1* and *SMN2*, which encode survival motor neuron (SMN) protein, are associated with ALS and recent evidence suggests that *SMN1* duplications confer increased risk of ALS. SMN normally interacts with TDP-43 at transcriptional and post-translational levels with TDP-43 regulating *SMN2* splicing and assembly of SMN nuclear complexes or gems in motor neurons. However, the effect of TDP-43 pathology on SMN expression and gem formation in ALS is unclear. We therefore examined SMN expression and nuclear complexes in transgenic TDP-43^A315T mice.

Objectives: To investigate SMN expression and nuclear complex formation in motor neurons of transgenic TDP-43[A315T] mice at different disease stages. To determine whether SMN is a modifier of mutant TDP-43 mediated ALS, we also crossed TDP-43[A315T] and SMN transgenic mice and analysed double transgenic mice.

Methods: SMN expression was analysed in presymptomatic (P30, 60) and symptomatic (P90) TDP-43[A315T] mice and age-matched wild-types using real-time PCR and immunoblotting. SMN nuclear complexes were also counted in spinal motor neurons of mice. TDP-43[A315T] mice were intercrossed with mice overexpressing human SMN driven by the prion promoter (PrP-SMN). Double transgenic TDP-43[A315T]; PrP-SMN mice and control genotypes TDP-43[A315T] and PrP-SMN were examined for weight loss, motor function and survival. Spinal cords and brains were analysed by motor neuron counts, SMN expression and nuclear complexes.

Results: SMN protein expression was increased by 1.5-fold and 2-fold, respectively, in spinal cords of presymptomatic and symptomatic TDP-43[A315T] mice. There was a corresponding accumulation of nuclear and cytoplasmic SMN complexes in spinal motor neurons of TDP-43[A315T] mice. Transgenic overexpression of SMN accelerated weight loss, motor deficits and death in double transgenic TDP-43[A315T];PrP-SMN mice compared to TDP-43[A315T] controls. This resulted from increased severity of motor neuron loss and excess nuclear and cytoplasmic SMN complexes in spinal cords.

Discussion and conclusion: Our results demonstrate that mutant TDP-43 triggers pathological accumulation of SMN and its nuclear complexes in spinal motor neurons, consistent with recent genetic association data showing that *SMN1* duplications increase susceptibility to ALS. Furthermore, forced overexpression of SMN compounds the disease phenotype of TDP-43[A315T] mice, suggesting that SMN is an enhancing modifier of TDP-43 pathology.

C47   CYCLOPHILIN A INTERACTION NETWORK PERTURBATION IS A CONVERGING PATHO-MECHANISM IN DIFFERENT FORMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TDP-43, SOD1, hnRNP

Background: Familial and sporadic amyotrophic lateral sclerosis (ALS) phenotypes are clinically indistinguishable. Cyclophilin A (CypA) is a translational biomarker of ALS: it is a hallmark of disease in mutant SOD1 (mSOD1) animal models and in sporadic patients (1-2). Moreover, CypA is enriched in the spinal cord aggregates of mSOD1 mice and sporadic ALS patients (3). CypA is an ubiquitous protein with multiple functions relevant to the central nervous system, where it is abundantly expressed.

Objectives: The aim of our study is to provide insights into the molecular function of CypA in familial and sporadic forms of ALS. In particular, starting from apparently unrelated forms of disease we provided the molecular basis for a converging clinical phenotype and offer hints for therapeutic strategies.

Methods: The CypA interactors were identified by a co-immunoprecipitation approach combined with 2D gel electrophoresis and MALDI TOF/TOF mass spectrometry in HEK293 cells stably expressing mSOD1. We validated the results in the SOD1-G93A mouse model and in lymphomonocytes from sporadic ALS patients. We tested the contribution of CypA to disease mechanism(s) performing functional analyses *in vitro* and *in vivo*.

Results: The proteomic analysis of CypA interacting proteins revealed that CypA functionally associates with different protein networks. In particular, it extensively binds proteins regulating RNA metabolism, including several hnRNPs and TDP-43. TDP-43 and CypA interact in the nucleus, in an RNA-dependent way. CypA has a key role in the stabilization of TDP-43/hnRNP A2/B1 interaction, and TDP-43-mediated HDAC6 expression regulation, properties impaired in TDP-43 ALS-mutants, possibly because of a loss-of-interaction with CypA. Interestingly, an altered CypA/TDP-43 interaction was observed also in lymphomonocytes of sporadic ALS patients, indicating that CypA interaction network perturbation is relevant also in sporadic forms, where it is aberrantly post-translationally modified. CypA interacts also with mSOD1, and mice expressing mSOD1 and lacking CypA show increased levels of insoluble mSOD1 and hyperphosphorylated TDP-43 in the spinal cord.

Discussion and conclusion: This work shows that CypA has a protective role in ALS: as a chaperone for mSOD1 and in maintenance of multi-protein (TDP-43/hnRNPs) complex dynamic nature. Regardless of the cause of the disease, CypA interaction network is perturbed and CypA may be sequestered in proteinaceous aggregates, altering its protective activities. The net effect is the development of TDP-43 proteinopathy that may lead to a compromised RNA metabolism. CypA being a key interacting partner of both mSOD1 and TDP-43 can represent the 'missing link' of seemingly divergent patho-mechanisms in ALS and an interesting target for therapeutic interventions.

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SESSION 6B NEUROIMAGING

C48   NEUROIMAGING IN ALS: CAN WE SEE MORE CLEARLY?

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The use of conventional magnetic resonance imaging (MRI) of the brain and spinal cord in patients suspected of having a motor neuron disease (MND) is recommended to exclude other causes of signs and symptoms of MN pathology. The detection of corticospinal tract (CST) hyperintensities on conventional MRI and a T2-hypointense rim in the precentral gyrus can support a pre-existing suspicion of MND. However, the specific search of these abnormalities for the purpose of making a firm diagnosis of MND is not recommended.

Over the past 10 years, there have been significant advances in the identification of advanced neuroimaging patterns in MND. A significant cortical thinning of the precentral gyrus, damage to the CST and corpus callosum assessed using diffusion tensor (DT) MRI, and altered N-acetylaspartate levels in the primary motor cortex and CST hold promise for assessing the upper motor neuron involvement before clinical symptoms become apparent. Importantly, DT MRI measures of the CST have a prognostic value in amyotrophic lateral sclerosis patients. Furthermore, patterns of brain damage are emerging to identify patients prone to develop dementia or have a rapid progression. It is strongly advisable to incorporate measures derived from advanced MRI techniques into new clinical trials as exploratory outcomes to gain additional insights into the value of these techniques in the assessment of MND in the individual patient and to validate them further in the context of multicenter, longitudinal studies.

C49   THE NEUROIMAGING SIGNATURE OF THE C9ORF72 HEXANUCLEOTIDE REPEAT IN AMYOTROPHIC LATERAL SCLEROSIS – A MULTIMODAL MRI STUDY

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Keywords: C9orf72 hexanucleotide repeat expansion, MRI, diffusion tensor imaging

Background: The main pathological and clinical features of the *C9orf72* hexanucleotide repeat expansion in Amyotrophic lateral sclerosis are currently being established. Population-based studies indicate that the clinical phenotype is associated with fronto-temporal dysfunction, younger age of onset and worse survival outcomes compared to C9orf negative ALS.

Objectives: To characterise the distinctive cortical and subcortical features of the *C9orf72* genotype in ALS in comparison to C9orf72 negative ALS patients, C9orf72 negative ALS-FTD patients and healthy controls.

Methods: A prospective, single-centre, single-protocol, grey and white matter magnetic resonance imaging study was undertaken with 30 *C9orf72* negative ALS patients, nine ALS patients carrying the *C9orf72* hexanucleotide repeat expansion, eight C9orf72 negative ALS-FTD patients and 44 healthy controls. Groups were carefully matched for disease duration, age and handedness. Voxel-based morphometry, cortical thickness analyses and tract-based spatial statistics were carried out for axial diffusivity, radial diffusivity, mean diffusivity and fractional anisotropy. All patients underwent comprehensive neuropsychological profiling and were screened for other mutations in genes previously implicated in ALS such as FUS, OPTN, SOD1, TARDBP, GRN, ANG, ATXN2.

Results: A congruent pattern of cortical and subcortical involvement was identified in those with the C9orf72 genotype, affecting fusiform, thalamic, supramarginal, orbitofrontal regions and Borca's area. The *C9orf72* negative cohort demonstrated the "classical" distribution of ALS pathology affecting primarily the motor cortex, motor pathways and cerebellar regions with strikingly limited extra-motor expansion. While the body of the corpus callosum and superior motor tracts were affected in both ALS genotypes, the anterior commissure and the genu of the corpus callosum showed *C9orf72* specific vulnerability.

Conclusions: Extensive cortical and subcortical frontotemporal involvement was identified in association with the *C9orf72* genotype in ALS, compared to the relatively limited extra-motor pathology in *C9orf72* negative patients. The distinctive, genotype-specific pathoanatomical patterns are consistent with the neuropsychological profile of the two ALS cohorts. Our findings also suggest that previously described extra-motor changes in ALS could be partially driven by the inclusion of patients with the *C9orf72* genotype. Given the very strong neuropathological signature of the *C9orf72* genotype, we suggest that future imaging, neuropsychology and pharmaceutical studies in ALS be stratified for the presence of this mutation.

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C50 A VISUAL MRI ATROPHY SCALE FOR THE ALS-FTD CONTINUUM

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Background: ALS and FTD overlap with some patients exhibiting clinical features of both diseases (ALSFTD). Early identification of ALSFTD remains challenging; however, recent imaging findings suggest that ALSFTD patients show significantly more cortical atrophy than ALS but less atrophy than bvFTD. Still these studies employed complex imaging techniques which are not suitable for a clinical setting.

Objective: To distinguish ALS, ALSFTD and bvFTD via a novel visual MRI cortical atrophy scale which can be employed in a clinical setting.

Methods: MRI images of 71 participants (22 ALS, 11 ALS-FTD, 24 bvFTD and 14 controls) were rated in four brain areas such as orbitofrontal cortex, anterior temporal lobe, anterior cingulate and motor cortex. Areas of atrophy were rated on a 5-point Likert scale by two raters blinded to the diagnosis.

Results: A continuum of atrophy scores emerged with bvFTD patients exhibiting the highest level of atrophy, while ALS patients had the lowest atrophy scores. ALSFTD patients had a higher motor cortical atrophy rating compared to ALS patients with statistical trends for more atrophy in ALSFTD for the anterior cingulate and anterior temporal lobe. ALSFTD patients could be distinguished from bvFTD patients on orbitofrontal cortex atrophy, with bvFTD being more severely affected.

Conclusion: Our study demonstrates that a simple visual MRI rating scale can reliably distinguish ALS, ALSFTD and bvFTD atrophy patterns in a clinical setting. Motor cortex, anterior cingulate and anterior temporal atrophy emerged as good diagnostic markers for ALSFTD, whereas orbitofrontal cortex atrophy was specific for bvFTD. Employment of this MRI rating scale can complement clinical diagnostics of patients in the ALS-FTD continuum.

C51 CEREBELLAR SUBSTRUCTURE INTEGRITY IN AMYOTROPHIC LATERAL SCLEROSIS AND BEHAVIOURAL VARIANT FRONTOTEMPORAL DEMENTIA

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Background: Emerging evidence implicating the human cerebellum in intact cognitive and behavioural processes has awakened a wide interest in this region, which was previously associated only with sensorimotor control. Recent structural findings across the ALS-FTD continuum found gross cerebellar atrophy in both ALS and bvFTD, suggesting a crucial role of this region to the overlapping cognitive and motor deficits that are increasingly recognised in ALS and bvFTD, respectively. However, there have been no studies to date assessing which cerebellar subregions are affected in ALS and bvFTD.

Objective: To contrast the grey matter integrity of cerebellar subregions in ALS and bvFTD.

Methods: Seventy-nine participants with ALS (n = 25) and behavioural variant FTD (n = 17) as well as controls (n = 37) underwent structural imaging using voxel-based morphometry (VBM) in FSL. Grey matter differences in the cerebellar lobules, vermis and crus were established using a region of interest (ROI) imaging approach of the probabilistic cerebellar atlas (1). All results are reported at $p < 0.05$ corrected for family-wise error (FWE) or false discovery rate (FDR) multiple comparison correction.

Results: Patients with ALS demonstrate widespread bilateral atrophy in the posterior lobules and only minor atrophy in the left crus in comparison to controls. By contrast, bvFTD patients revealed only minor atrophy changes in the right anterior lobules, but substantial bilateral atrophy in the crus. Direct comparisons between patient groups confirmed this dissociation, with more severe atrophy in the posterior lobules in ALS, and significant changes in the crus and anterior lobules in bvFTD. The vermis was not affected in either disease.

Discussion: We demonstrate here for the first time a structural dissociation between cerebellar subregions affected in ALS and bvFTD, with posterior lobules being affected in ALS and anterior lobules and crus being affected in bvFTD. These results are also corroborated by functional neuroimaging in the healthy showing the posterior lobule being critical for motor control (2), while the right anterior lobule and bilateral crus are activated for behavioural and emotions correlates (3-5). Interestingly, the cerebellar vermis, recently described as being a recipient of dense motor cortical afferents (6), was not affected in either ALS or bvFTD. Overlap in cerebellar substructures may underlie the cognitive and motor deficits observed across the ALS-FTD continuum, which future studies need to investigate.

Conclusion: Grey matter integrity in cerebellar substructures distinguishes between ALS and bvFTD.

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C52   PROTON MRSI OF CEREBELLUM IN ALS

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Keywords: cerebellum, whole-brain MRS, upper motor neuron (UMN)

Background: ALS is a neurodegenerative disorder that primarily affects the motor system, which is composed of motor and supplementary motor cortices, spinal cord, basal ganglia and cerebellum. The pathological (1,2) and functional MRI studies (3) have demonstrated the involvement of cerebellum in patients with ALS. A limited number of *in vivo* studies of DTI (4,5) and MRS (6) in patients with ALS have revealed variable results of significant (5) to no involvement of cerebellum (4,6).

Objectives: To assess the involvement of cerebellum in patients with ALS using proton MRS method.

Methods: Thirty-seven definitive-ALS patients (50 ± 7 (SD) years) and 37 age-matched controls were scanned on a 3T scanner using a whole-brain MRSI sequence (FOV: $280 \times 280 \times 180$ mm^3, $50 \times 50 \times 18$ phase encodes, slab thickness of 135 mm, TR/TE $= 1710/70$ ms, and 26 min acq time). The details of data acquisition and processing are published (7). The data from cerebellar hemispheres were obtained to evaluate N-acetyl aspartate (NAA), creatine (Cre), choline (Cho), and ratios among them. The normalized metabolite data were compared using ANCOVA and a p-value of less than 0.05 was considered significant. None of the patients had clinical evidence of extra-motor involvement. The clinical neurological assessments included percentage maximum forced vital capacity (FVC), ALSFRS-R, quantification of upper motor neuron function (foot tap, finger tap, lip and tongue movement rate (7)).

Results: The major findings included lower NAA (13213 ± 181 (SE) vs. 13771 ± 181; p $= 0.03$), higher Cho (3074 ± 67 vs 2801 ± 67; p $= 0.005$), lower Cre (13265 ± 212 vs 14434 ± 212; p $= < 0.0001$), lower NAA/Cho (4.66 ± 0.15 vs 5.13 ± 0.15; p $= 0.028$), lower NAA/Cre (0.95 ± 0.02 vs 1.11 ± 0.02; p $= 0.028$) in patients compared to controls. There were mild to moderate correlations (r $= 0.3$–0.5; p $= 0.04$–0.003) between the metabolite and various clinical measures.

Conclusions: The lower NAA, Cre, NAA/Cho and NAA/Cre, and higher Cho in the cerebellum of patients with ALS are indicative of neuronal loss or dysfunction and alterations in the choline containing membranes.

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C53   DISCRIMINANT VALUE OF 18FDG-PET IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: PET, sensitivity, specificity

Background: Amyotrophic lateral sclerosis (ALS) is characterized by the loss of spinal and bulbar motor neurons and corticospinal tracts degeneration. A disease marker of upper motor neuron (UMN) degeneration is still lacking. Studies on [1]H-MR spectroscopy and DTI hold promise for detecting and quantifying subclinical UMN damage. Few data have been reported on cerebral [18]FDG-PET.

Objective: To assess the discriminant value of [18]FDG-PET at rest in the largest series of ALS patients investigated so far.

Methods: A total of 195 ALS patients (77 women and 118 men, mean age: 63 (SD 12)) and 40 control subjects (11 women and 29 men, mean age 62 (SD 14)) underwent brain [18]FDG-PET. Twenty-six cortical and sub-cortical brain regions were segmented by the Pick Atlas tool in SPM2 and relative metabolic uptakes individually normalized by whole brain values. Factorial (FA) and Discriminant (DA) analysis were performed using the 52 bilateral regions as well as age and sex. The strict statistical constraint of post-hoc cross-validation was applied.

Results: FA identified eight factors in CTRL and 10 factors in ALS explaining 90% and 87% of total variance, respectively. As compared to CTRL, ALS showed factors with stronger laterality, networking separately left and right temporal lobes and left somatosensory and superior parietal cortex. In ALS FA gathered also selectively insulas and central

structures (caudate and thalamic nuclei). DA performed on all 235 subjects showed following cross-validation an accuracy of 88% (sensitivity 89% and specificity 83%) when all 52 regions were taken into account and of 80% (equal sensitivity and specificity) when analyzing the discriminant value of the 10 factors.

Conclusions: In ALS different cortical and subcortical networks were found as compared to normal controls. When 52 functional regions were submitted to discriminant analysis the overall accuracy was 88% with a very high sensitivity of 89%. These findings show for the first time the usefulness of ^{18}FDG-PET in differentiating ALS patients from normal controls and pave the way to a larger implementation of such methodology in ALS diagnosis.

C54   DEVELOPMENT OF A PET RADIOLIGAND FOR THE NON-INVASIVE IMAGING OF CANNABINOID TYPE 2 RECEPTOR

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Keywords: cannabinoid receptor type 2 ligand, Positron Emission Tomography

Background: In amyotrophic lateral sclerosis (ALS) post-mortem tissue and in mice the Cannabinoid type 2 receptor (CB2) receptor is upregulated. Manipulation of the endocannabinoid system via activation of the CB2 in ALS animal models has consistently shown neuroprotective effects (1-3). However, so far no radioligand is available which would enable to study this upregulation *in vivo* by Positron Emission Tomography (PET).

Objectives: To develop a selective CB2 radioligand, we selected one of 4-oxoquinoline derivatives (designated KD-2) as a potential PET tracer for imaging CB2.

Methods: KD-2 and its corresponding precursor for radiolabeling have been synthesized according to the published procedure (4). The radiosynthesis of [^{11}C]KD-2 was accomplished in a one-step reaction sequence starting from a phenolic precursor and [^{11}C]methyl iodide. *In vitro* studies included transport experiments across a blood-brain barrier model (P-glycoprotein-transfected MDCK cells) and autoradiography with rodent spleen samples, a tissue with high CB2 levels and post-mortem spinal cord of ALS patient. [^{11}C]KD-2 was further evaluated *in vivo* in the rat by PET under baseline and blocking conditions.

Results: [^{11}C]KD-2 (ca. 3-5 GBq) was obtained in 99% radiochemical purity after semi-HPLC purification. *In vitro* barrier permeation of [^{11}C]KD-2 was in the range of blood-brain barrier (BBB) permeating compounds. No efflux by P-glycoprotein was detected. *In vitro* autoradiography with rat and mouse spleen slices demonstrated high-specific binding towards CB2. High spleen uptake of [^{11}C]KD-2 was observed by PET with Wistar rats and its specificity was confirmed by displacement with the selective CB2 agonist GW405833. A pilot autoradiography study with post mortem spinal cord tissues from ALS patients showed specific binding of [^{11}C]KD-2, suggesting the presence of significant levels of CB2 in spinal cord of ALS patients.

Discussion and conclusion: [^{11}C]KD-2 shows good *in vitro* and *in vivo* properties as a potential PET tracer for CB2. CB2 imaging by PET may become of interest for diagnosis and monitoring of disease progression and therapy success in ALS.

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SESSION 7A GENETICS AND GENOMICS

C55   REDUCED C9ORF72 GENE EXPRESSION IN C9FTD/ALS IS CAUSED BY TRIMETHYLATION OF HISTONE H3K9

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Keywords: C9ORF72, epigenetic modifications, methylation

Individuals carrying expanded repeats in the *C9ORF72* gene represent a significant portion of patients suffering from amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Elucidating how these expanded repeats cause "C9FTD/ALS," and the associated reduction in *C9ORF72* transcript expression, has since become an important goal of the field. As such, we sought to investigate whether epigenetic changes, such as methylation of histones and repeat sequences, and hypermethylation of neighboring islands rich in cytosine-phosphate-guanine (CpG) which lead to gene silencing and consequently loss of function in other repeat disease play a similar role in C9FTD/ALS. We treated fibroblasts obtained from C9FTD/ALS patients with 5-aza-2-deoxycytidine (5-Aza), and compared C9ORF72 mRNA and protein expression levels in treated and untreated cells. We also performed chromatin immunoprecipitation (ChIP) as well as bisulfite modification of genomic DNA to respectively evaluate histone and CpG island methylation. Moreover, we analyzed mRNA and protein expression levels as well as DNA and histone methylation in the frontal cortices and cerebella of postmortem C9FTD/ALS cases. After confirming reduced levels of C9ORF72 mRNA and protein levels in C9FTD/ALS, we showed that the 5-Aza treatment increased expression. We also demonstrated that CpG islands flanking the repeat expansion were not methylated in fibroblasts and brain tissues. More important, we discovered that histone H3 at lysine 9 (H3K9) was trimethylated, an event known to repress gene expression, in all pathogenic repeat carriers. Taken together, our results clearly demonstrate that trimethylation of histone H3K9 is the mechanism involved in reducing the expression of C9ORF72 mRNA and protein in expanded repeat carriers, and may be a promising target for therapeutic intervention. Given that therapies targeting DNA/histone methylation are currently being developed for use in cancer patients, the application of such therapies, in combination with histone deacetylase inhibitors, may be a promising therapeutic strategy for C9FTD/ALS patients.

C56   EXTENSIVE SOUTHERN BLOT STUDY OF C9ORF72 EXPANSION CARRIERS

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Keywords: C9ORF72, repeat expansion size

Background: Hexanucleotide repeat expansions in chromosome 9 open reading frame 72 (*C9ORF72*) are the major genetic cause of frontotemporal dementia (FTD) and motor neuron disease (MND). These expansions can harbor hundreds to thousands of GGGGCC repeat units. Repeat-primed PCR methods are able to assess the presence or absence of *C9ORF72* expansions; however, they are unable to determine the actual repeat size, as opposed to Southern blotting techniques. To date, large scale Southern blot studies that investigate *C9ORF72* repeat sizes in brain tissue have not been reported, and hence, it is currently unknown whether these repeat sizes affect disease severity or phenotypes.

Objectives: To determine repeat sizes in a large cohort of *C9ORF72* expansion carriers and to correlate repeat sizes with patient characteristics.

Methods: We performed an extensive Southern blot characterization study in a cohort of 84 *C9ORF72* expansion carriers, including FTD patients (n = 35), FTD/MND patients (n = 16), MND patients (n = 30), and unaffected subjects (n = 3). More than 200 independent DNA samples obtained from frontal cortex, temporal cortex, parietal cortex, occipital cortex, spinal cord, cerebellum, blood, skin-derived fibroblasts, spleen, heart, muscle, pancreas, liver, and testes were investigated. A total of 7–10 µg of genomic DNA was used for Southern blotting.

Results: Repeat lengths in the cerebellum were significantly smaller (median ~1667 repeat units) than in the frontal cortex (median ~5250 repeat units, $p < 0.001$), or in blood (median ~2717 repeat units, $p < 0.001$). Within these tissues, there was no significant difference in repeat length between disease subgroups, nor did we detect associations with gender, TDP-43 type, family history, or size of the wild-type allele. Individual patients did demonstrate substantial variation in repeat length across tissues, such as blood and fibroblasts. In the frontal cortex of FTD patients, age at onset strongly correlated with repeat length ($r = 0.63$, $p = 0.003$) and smear size ($r = 0.66$, $p = 0.002$). Finally, in the cerebellum, survival after disease onset was poorer in patients from our overall cohort with repeat lengths greater than 1467 repeat units (RR 3.28, $p = 0.010$), corresponding to a decrease in survival of ~2.5 years.

Discussion: *C9ORF72* repeat expansions cause a range of neurodegenerative phenotypes. At the moment, it is impossible to predict disease onset, progression, and/or manifestations of *C9ORF72* expansion carriers. Our present study has revealed significant variability in *C9ORF72* repeat sizes across tissues and patients. In the frontal cortex of FTD patients, repeat sizes were relatively unstable and age-dependent; long repeat sizes in the cerebellum conferred an important survival disadvantage. Repeat sizes, however, did not predict disease phenotype, and were not associated with other clinical or pathological features. Based on our findings, only repeat sizes in the cerebellum, a region unaffected by neuronal loss, could provide reliable information on disease severity, which is highly relevant for genetic counseling.

C57   C9ORF72 GGGGCC EXPANDED REPEATS PRODUCE SPLICING DYSREGULATION WHICH CORRELATES WITH DISEASE SEVERITY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: transcriptome, C9ORF72, splicing

Background: Expanded GGGGCC repeats in intron 1 of the *C9ORF72* gene represent the most common cause of familial amyotrophic lateral sclerosis (ALS). Other neurodegenerative diseases result from expanded repeat sequences in non-coding regions raising the possibility of a common pathogenic mechanism. In particular, toxic gain-of-function by sequestration of RNA-binding proteins (RBPs) is a major factor underlying myotonic dystrophy types 1/2 (DM1/2). If *C9ORF72* expansions mediate pathogenesis by a similar mechanism then a global disruption of splicing might be expected.

Objective: To utilise an exon level transcriptome analysis to characterise the effect of *C9ORF72* expansion on global splicing patterns.

Methods: Lymphoblastoid cell lines derived from ALS patients (n = 54) and neurologically normal controls (n = 15) were obtained. Extracted RNA was assessed using Human Exon 1.0ST GeneChip® microarrays. Data were analysed using the 'finding isoforms using robust multichip analysis (FIRMA) package. Highly negative or positive values of the FIRMA score are indicative of alternative exon skipping or inclusion, respectively. Consistency of splicing within a sample group such as patients or controls was evaluated by comparing the number of splicing events which occurred in 1, 2, 3, … n samples within the group. To allow comparison between groups, reference was made to a model in which exons are spliced in or out at random.

Results: There was no difference in the total number of splicing events observed in lymphoblastoid cell lines derived from *C9ORF72+* patients, *C9ORF72-* patients and controls. However, the nature of those splicing events was significantly different. It is expected that functionally appropriate splicing would be similar in samples of a particular group. Consistency was significantly reduced in the *C9ORF72+* group compared

to *C9ORF72-* patients and controls. In addition, splicing was less consistent in *C9ORF72+* patients who lived less than 2 years compared to those that lived more than 5 years.

Discussion and conclusion: These data are consistent with sequestration of RBPs by the *C9ORF72* expansion which would significantly impact the splicing machinery of the cell. We suggest that reduction of splicing consistency reflects an increased splicing error rate which may, over time, lead to a crucial pathogenic splicing event(s). This might explain both the variability and late age of onset of *C9ORF72*-ALS. Consistent with this those patients with the lowest consistency of splicing had more severe disease.

C58   MOTOR NEURON SPECIFIC TRANSLATIONAL PROFILING IN SOD1G93A TRANSGENIC MICE

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Keywords: translational profiling, TRAP, motor neuron

Background: Previous profiling studies in cell and transgenic mouse models of ALS have provided large lists of genes and pathways that are potentially relevant to the disease mechanisms causing motor neuron degeneration. However, the mRNA isolation techniques used in these studies have been hampered by challenges such as lack of cell specificity or disruption of native cellular environment.

Objectives: To overcome these disadvantages and determine the translational changes occurring specifically in the spinal cord motor neurons of ALS, we employed a novel technique called Translating Ribosome Affinity Purification (TRAP).

Methods: TRAP utilizes BAC transgenic mice expressing an EGFP-tagged ribosomal protein, L10a, in genetically targeted cell populations via use of cell-specific gene promotors. TRAP facilitates immunoaffinity purification of EGFP-tagged polysomes and bound mRNAs from genetically determined cell populations, thereby combining coincident detection of cell-type specificity and all translated mRNAs *in vivo*.

SOD1[G93A] (±) mice were crossed with *ChAT* bacTRAP (±) mice, which express EGFP-L10a exclusively in cholinergic neurons, including motor neurons. Spinal cords were dissected from SOD1[G93A] (±): *ChAT* bacTRAP (+) mice and

control *ChAT* bacTRAP ($\pm$) littermates at pre-symptomatic stage (10 weeks). mRNAs isolated using TRAP were identified on an Agilent G3 Mouse GE *8 x 60K microarray*.

Results: Bioinformatics analyses revealed 75 transcripts differentially expressed in SOD1[G93A] ($\pm$):*ChAT* bacTRAP ($\pm$) mice compared to control. Of 51 known genes 29 were up-regulated and 22 down-regulated. These genes have been documented to be involved in numerous biological processes. Immunohistochemistry confirmed two genes, 3-phosphoglycerate dehydrogenase (Phgdh) and anaphase-promoting complex subunit 1 (Anapc1), displayed the most profound changes at the protein levels. Phgdh, an enzyme critical for serine biosynthesis, was dramatically induced in the degenerating motor neurons of SOD1[G93A] ($\pm$) mice in contrast to control where it was absent in motor neurons, consistent with previous observations in SOD1[G37R] and SOD1[G85R] transgenic mice using LCM (1). Anapc1, a component of the cycosome regulating mitosis progression, displayed a subcellular mislocalization from predominantly nuclear to cytoplasmic in motor neurons of SOD1[G93A] ($\pm$) mice. A phosphorylated Anapc1, pSer377-Anapc1, displayed similar changes in degenerating motor neurons of SOD1[G93A] ($\pm$) mice as well as all familial and sporadic ALS cases examined.

Discussion and conclusion: Together, using the TRAP technique, we have for the first time identified specific translational changes occurring in spinal cord motor neurons of 10-week SOD1[G93A] transgenic mice. Our findings of Phgdh and Anapc1 protein expression changes in ALS-linked degenerating motor neurons have advanced our understanding of previously less explored pathways, ie serine biosynthesis and cyclosome function, which may be involved in the mechanisms causing ALS. We expect this work will ultimately lead to the development of biomarkers and/or effective therapeutics for this devastating disease.

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C59   MORE EVIDENCE SUPPORTING PERTURBATION IN EXTRACELLULAR AND TRANSMEMBRANE DOMAINS AND OF PROTEIN SIGNALING BY TRANSCRIPTOME ANALYSIS OF MOTOR NEURONS FROM SPORADIC ALS SPINAL CORDS

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Keywords: genomics, pathology, transcriptome

Background: While many of the ALS genes play an important role in RNA-related processes, how mutations lead to temporally spatially progressive motor neuron degeneration remains unclear. We have previously shown that the focal onset and regional spread of motor neuron dysfunction can leave motor neuron-rich regions in post-mortem spinal cords and that transcriptome signatures can be clearly resolved.

Here we continue this work with RNA-seq technologies and new bioinformatic approaches.

Objectives: Our objectives were to illuminate key transcriptional signatures from residual motor neurons in neuron-rich regions using RNA-seq and to seek biological enrichments.

Methods: We used used laser-capture microdissection to selectively enrich RNA pools with motor neurons in 13 sALS and nine control spinal cords. cDNA libraries were generated from each spinal cord and RNA-seq was performed to obtain a whole-transcriptome dataset of gene expression levels from each replicate. We used BiomarkTM HD System from Fluidigm® for high-throughput qPCR validation.

Results: Traditionally, disease samples would be compared to control samples in a pairwise fashion, providing a list of significantly changed genes for each comparison. Owing to the high level of variation found not just between individual patients, but also because of the unknown shared (or unshared) genetic factors underlying sALS patients, a unique analysis method was devised to focus on commonly changing genes despite the high level of noise, termed Median Percentile Rank (MPR). This analysis, meant to avoid biases in normalizing ratios (Buck, Lieb 2004), works as follows. Each of the 117 lists of gene expression fold-changes is sorted, and a rank is assigned. The percentile rank is calculated from a gene's position in this list, and the median of a gene's 117 percentile ranks is calculated, giving the MPR statistic. A histogram can then be constructed from these MPR values, and a clear threshold can be drawn near the two edges of the histogram if there is enrichment above expected.

From this analysis, 259 to more than 2,346 and 286 to more than 955 genes (strict to non-strict cutoffs) were found to be up- and down-regulated, respectively. Gene set enrichment analysis revealed results for the up-regulated genes, with enrichment for GO terms extracellular region ($p < $E-59), signal peptide ($p < $E-59), disulfide bond ($p < $E-68), glycoprotein ($p < $E-62), and membrane-related functions ($p < $E-24). In addition, defense/inflammatory response ($p < $E-30) was identified. Interestingly, the down-regulated set showed no confident results. To validate these changes, 25 up-regulated and 15 down-regulated genes were chosen to be analyzed across all 22 samples (13 sALS and 9 controls).

Conclusion: These results identify significantly perturbed transcriptional programs related to extracellular and membrane domains and to protein signaling, either contributing to or resulting from motor neuron degeneration in sALS. They are identified by unbiased discovery methods and have been relatively unemphasized.

C60   TRANSLATIONAL STUDY OF POTENTIAL PROGNOSTIC AND DIAGNOSTIC BIOMARKERS TO HUMAN SAMPLES

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Keywords: muscle biopsy, lymphocyte, prognostic factors

Background: The search of biomarkers in ALS is being carried out on post-mortem patient's samples, such as brain or spinal cord, or on samples invasively obtained, such as cerebrospinal fluid (CSF) and more recently mesenchymal stem cells from bone marrow (1). Growing tendency relies on the study of new tissues that can be analyzed in a less invasive way (2). Previous studies in our group suggested five genes, *Mef2c, Gsr, Col19a1, Calm1* and *Snx10*, as potential genetic biomarkers of longevity in the animal model for ALS (3). We translated this study to human samples from skeletal muscle and blood to validate the potential nature of these biomarkers.

Objectives: The main aim of this study was to identify potential prognostic and diagnostic biomakers in muscle biopsies and blood samples from ALS patients.

Methods: Muscle biopsies proceeded from brachial biceps. The lymphocyte fraction from total blood was isolated using Ficoll gradient. Real time PCR and western blot were used to analyze all the samples in the 17 genes previously selected. ANOVA and Kruskal Wallis test were used to compare means. ROC curves were calculated to study the diagnostic criterion.

Results: In muscle biopsies, the results suggested that transcriptional and transductional levels of *Col19a1* could be used as a diagnostic biomarker for ALS. Regarding lymphocyte samples, transcriptional levels of *Col19a1* could also be used as a diagnostic biomarker. Moreover, *Col19a1, Impa1, Mef2c, Nogo A, Snx10, Gsk3* and *Gsr* could be considered as potential prognostic biomarkers of the disease.

Discussion: In this study, translational research work has been made from transgenic SOD1[G93A] mice to muscle biopsies and lymphocytes from ALS patients. Seventeen genes were validated in human samples. Interestingly, transcriptional and transductional expression of *Col19a1* was significantly increased and specifically linked to ALS patients, yielding a positive predictive value of 100% and a negative predictive value of 99,998%. Regarding lymphocyte samples, transcriptional expression of *Col19a1* significantly increased and was specifically linked to ALS patients, considering this gene as a potential diagnostic biomarker. Regarding the rest of genes, *Impa1, Mef2c, Nogo A, Snx10, Gsk3, Gsr* and also *Col19a1* were identified as potential prognostic biomarkers of the disease in lymphocytes from ALS patients.

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SESSION 7B BIOMARKERS

C61 SERUM CREATININE, A BIOMARKER FOR MUSCLE MASS IN AMYOTROPHIC LATERAL SCLEROSIS(ALS), PREDICTS LOSS OF AMBULATION MEASURED BY ALS FUNCTIONAL RATING SCALE-REVISED WALKING ITEM SCORE(ALSFRSW)

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Keywords: biomarker, creatinine, disease progression

Background: Serum creatinine has been identified as a muscle mass biomarker related to the diagnosis and rate of progression of ALS and bulbar spinal muscular atrophy (Kennedy's disease) in French, Japanese and American ALS patients (1-5). Recent analysis of clinical staging in ALS patients has identified increased burden of leg involvement in El Escorial Criteria-Clinically Definite ALS patients (6).

Objectives: To define the relationship between serum creatinine and ALSFRSRw cross-sectionally and longitudinally over time in a clinic-based ALS patient population at Carolinas Neuromuscular/ALS-MDA Center.

Methods: Serum creatinine at first and subsequent clinic visits was related to ALSFRSR total score and ALSFRSRw from 2010 to 2012 in 311 ALS patients (183M and 128F; 55.7 ± 12.7 (SD) years). Cross-sectional analysis of all data as well as change in serum creatinine and ALSFRSRw between first and subsequent visits was analyzed with statistical software (MedCalc Software, Ostend, Belgium www.medcalc.org/).

Results: Serum creatinine cross-sectionally decreased significantly with decreasing ALSFRSw category (Creatinine = 0.54 (95% CI: 0.49-0.58) + 0.09(95% CI: 0.07–0.11) • ALSFRSRw ($R^2 = 0.2082$: $P < 0.001$)). Moreover, in individual ALS patients, change in serum creatinine decreased proportionate to decrease in ALSFRSRw (DeltaCreatinine = -0.11 (95% CI: -0.16 to 0.05) +0.08(95% CI: 0.04– 0.12)• ALSFRSRw ($R^2 = 0.1506$: $P < 0.001$)).

Discussion and conclusion: Decrease in serum creatinine significantly predicted change in ambulation measured by ALSFRSRw longitudinally. Further analysis is required to determine whether leg function alone and leg muscle mass are the major determinants of serum creatinine in ALS patients over the course of the disease.

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C62 MISFOLDED SOD1 IN BLOOD PLASMA IS AN ANTIBODY-ACCESSIBLE BIOMARKER FOR SPORADIC ALS

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Keywords: SOD1, biomarker, diagnosis

Background: ALS pathology is often associated with protein misfolding and aggregation. Clinical and pathological similarities between all forms of ALS suggest the existence of a common pathogenic pathway. Misfolded Cu/Zn superoxide dismutase (SOD1) has been increasingly identified in SALS and non-SOD1 FALS (1–3). There is increasing evidence that wild-type (wt) SOD1 may play a role in SALS, which in its misfolded form acquires many of the same cytotoxic properties as mutant SOD1 (4–6). Thus, misfolded SOD1 is a likely candidate for a common ALS diagnostic biomarker.

Objective: To determine if misfolded SOD1 is detectable in blood plasma from ALS patients using a novel diagnostic testing platform.

Methods: The mouse monoclonal antibody (mAb) 3H1 specifically binds to misfolded SOD1 and was used to perform immunoprecipitation experiments from 27 SALS and 27 normal control plasma samples. There was a significantly higher level of misfolded SOD1 detected in ALS compared

to normal plasma samples (p = 0.0001333). However, immunoprecipitation is a cumbersome technique that is not easily used for diagnostic testing. We have developed a simple dual-bead immunoassay that detects misfolded SOD1 in spinal cord from SOD1 G93A mutant mice and from two individuals with SALS, but not from a neurological control.

Results: Levels of misfolded SOD1 are approximately 35-times higher in G93A spinal cord than in SALS spinal cord. An anti-SOD1 rabbit mAb was generated with $EC_{50} = 35$ pM against misfolded SOD1, which is 10–100 fold higher than observed with mouse mAbs. This antibody specifically detects misfolded SOD1 in G93A mouse and human ALS spinal cord by immunohistochemistry, and control plasma spiked with G93A mouse spinal cord homogenate via the dual-bead immunoassay.

Conclusions: We have developed a mouse mAb that specifically detects misfolded SOD1 in SALS patient plasma via immunoprecipitation. Application of this antibody in a dual bead immunoassay specifically detects misfolded SOD1 in both ALS mouse and human spinal cord homogenate. A high-affinity rabbit mAb based on the same disease-specific epitope detect misfolded SOD1 in normal plasma spiked with G93A spinal cord homogenate. Further immunoassay development continues with ALS patient tissue and plasma samples.

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C63   PROTON NMR SPECTROSCOPY METABOLOMICS IN SERUM AND CSF

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Keywords: biomarker, metabolomics, spectroscopy

Background: Candidate 'wet' biomarkers are urgently sought in ALS. Results from agnostic proteomic approaches have not yet been replicated independently. Metabolomic studies using the non-selective technique ^{1}H NMR spectroscopy have reported serum glutamate as a marker of disease duration (1) and markers in CSF consistent with altered glucose metabolism (2).

Objectives: To determine whether there is a 'signature' of serum or CSF metabolites common to a large group of ALS patients.

Methods: The Oxford Study for Biomarkers in MND (Bio-MOx) obtained baseline serum and CSF samples in up to 70 patients across a range of initial disability and progression rates, with six-monthly longitudinal collection where possible, for up to two years. Samples were processed within one hour of extraction and centrifuged prior to storage in polypropylene at −80°C. Aliquots (100μL CSF or 150μL ultracentrifuged serum) were mixed with phosphate buffer in D_2O containing 1mM TSP as an internal standard (final volume 0.6mL). ^{1}HNMR spectra were acquired using a 700MHz Bruker spectrometer. Partial least squares discriminant analysis (PLS-DA) was used to determine differences between the spectra from patients and control volunteers, and according to progression rate. q^2 values above 0.4 were deemed to be significant.

Results: When comparing CSF spectra from age-matched control volunteers with the most advanced of the longitudinal samples (≥ 12 months from study enrolment), a significant separation was seen ($q^2 = 0.49$; 20 ALS and 17 control cases). This separation was still evident when less advanced patient samples were included (≥ 6 months from study enrolment, $q^2 = 0.45$; 26 ALS and 17 control cases), but not when the most advanced samples from each patient were modelled against the control cohort (i.e. including some baseline samples, $q^2 = 0.22$; 47 ALS and 17 control cases). No significant separation was seen with serum.

Discussion and conclusion: CSF proton NMR spectroscopy has potential for distinguishing ALS patients from healthy controls. The process of defining the key metabolites responsible for this separation is underway, and it is possible they will reflect systemic processes associated with the later stages of disease. Further model-building using multilevel PLS-DA will be used in an attempt to phenotypically stratify patients.

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C64   BETA-BAND INTERMUSCULAR COHERENCE AS A BIOMARKER OF UPPER MOTOR NEURON DYSFUNCTION IN MOTOR NEURON DISEASE

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Keywords: biomarker, upper motor neuron, coherence

Background: It has previously been demonstrated that beta-band intermuscular coherence (IMC) is a potential biomarker of upper motor neuron dysfunction (1), and does not change significantly during healthy adult life (2).

Objective: To investigate beta-band IMC in patients with motor neuron disease (MND) at first presentation to a specialist MND service.

Methods: We recruited 70 patients with possible, probable and definite MND (according to Awaji criteria) from the local tertiary MND service. Lower limb IMC was measured between a calf muscle and an intrinsic foot muscle during an unrestrained ankle dorsiflexion task. Upper limb IMC was similarly estimated between a forearm muscle and an intrinsic hand muscle during performance of an auxotonic precision grip task. Average beta-band IMC was computed for each limb and subject. Control data from 92 normal volunteers (age: 22–77) were available from our previous study. The probability distributions of MND and control data were modelled by variable kernel density estimates, and these estimates used to construct receiver operator characteristic (ROC) curves.

Results: The cumulative probability distribution of beta-band IMC in MND was similar in shape to that in normal controls but was shifted towards lower IMC values. The area under the ROC curve was approximately 75% for each limb.

Conclusion: Beta-band IMC represents an easily tolerated and inexpensive method for electrophysiological assessment of patients with MND. Our previous work suggests that the observed abnormalities are explicable in terms of upper motor neuron dysfunction. Upper motor neuron abnormalities have thus far proven difficult to detect in early MND, and beta-band IMC has potential as a semi-quantitative, clinically applicable biomarker with early sensitivity.

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C65   TRANSGLUTAMINASE 6 ANTIBODIES IN THE SERUM OF PATIENTS WITH ALS – IS GLUTEN SENSITIVITY INVOLVED IN MOTOR NEURON DEGENERATION?

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Keywords: gluten sensitivity, celiac, transglutaminases

Background: Celiac is an autoimmune disease in which the immune system attacks the inner wall of the intestine by a hypersensitivity reaction to gluten in genetically predisposed individuals. Transglutaminase 2 (TG2) is the primary autoantigen and IgA anti-TG2 production is used for diagnosis, as well as antibodies to deamidated gliadin peptide (DPG) and endomysium. Almost all patients with celiac disease present specific HLA genotypes (HLA -DQ2 encoded by DQA1*05 and DQB1*02 alleles or DQ8 encoded by DQB1*03:02).

Gluten sensitivity can cause extra-intestinal manifestations including neurological syndromes, commonly ataxia and neuropathy, with or without gastrointestinal symptoms. Most patients with gluten ataxia produce antibodies towards the newly identified neuronal transglutaminase 6 (TG6).

Two independent case reports described patients initially diagnosed with amyotrophic lateral sclerosis (ALS) and ultimately with celiac. Both started a strict gluten-free diet with improvement of symptoms during the following months.

Objectives: To evaluate the incidence of celiac-related and TG6 antibodies and HLA genotypes in ALS patients and controls, in order to evaluate whether a neurological presentation of gluten sensitivity mimicking ALS might occur occasionally.

Methods: We measured serum levels of IgA antibodies to TG2 and endomysium, IgG antibodies to DGP, IgA and IgG antibodies to TG6 in a cohort of patients with ALS and a group of healthy individuals of similar age and gender.

Results: We examined 149 patients (98 men, age 61.7 ± 12.3 years) and 114 controls (80 men, age 61.05 ± 11.5). All patients and controls were negative to IgA antibodies to endomysium and TG2. Sixty patients and 24 controls were tested for DGP antibodies and all except one were negative. Remarkably, 23 (15%) patients were positive to TG6 IgA antibodies as compared to only 5 (4%) controls (p = 0.0037). Three patients had borderline levels of TG6 antibodies.

The seropositive TG6 patients showed a classical picture of ALS with 75% males, age at disease onset 57.6 ± 12.5 years, 3 (13%) with bulbar onset, 19 (83%) patients had both upper and lower motor neuron involvement and a usual rate of progression, eight patients died or performed tracheostomy 24.5 ± 25 (range: 7–86) months after disease onset.

Fifty-one patients were tested for association with celiac specific HLA alleles. 73% of the TG6 IgA positive patients were positive to DQB1 as compared to only 44% of the TG6 IgA negative patients. The DQA1 alleles were carried by 74% of the TG6 IgA positive patients and 60% of the TG6 IgA negative patients. HLA typing in control individuals of similar ethnic origin is ongoing and will be reported at the meeting.

Conclusion: These preliminary data indicate that in certain cases ALS might be related to autoimmunity and gluten sensitivity. As gluten sensitivity is potentially treatable, this diagnostic challenge should not be overlooked.

C66   EFFECT OF LIPID PROFILE ON PROGNOSIS IN THE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: lipids, prognosis, high fat diet

Background: Patients with amyotrophic lateral sclerosis (ALS) are particularly predisposed to malnutrition for a variety of reasons which include dysphagia, fear of choking and aspiration, inability to feed themselves and high resting metabolic rate. Hence various defence mechanisms are likely to

be activated in such patients to provide energy substrates, for example, gluconeogenesis, lipolysis and ketogenesis. Higher weight in ALS is associated with a better outcome and it has been reported that patients with raised LDL/HDL ratio have a significantly improved survival. However, an opposing view is that body mass index and not dyslipidaemia is an independent predictor of survival in ALS. In common with the normal population of a similar age, an abnormal lipid profile is commonly seen in patients with ALS. An obvious explanation might relate to the mounting of a defence mechanism to provide energy substrates. Another possible explanation is that it might result from metabolic dysregulation or mitochondrial and/or endoplasmic reticulum (ER) stress. Mitochondrial and ER stress have been implicated in the pathogenesis of ALS. If it is a defence mechanism, then patients with a raised lipid profile may demonstrate a better prognosis. If it is a reflection of mitochondrial/ER dysfunction, then it could be associated with a poor outcome.

Objectives: To determine: (1) the prevalence (by gender) of raised lipid profile (cholesterol, LDH and triglycerides) in a large cohort of patients with ALS. (2) The relationship of lipid profile with the body mass index (BMI) through the ALS disease course. (3) Whether hyperlipidaemia develops with the progression of the disease or may be an early observation? (4) The implications (if any) of a raised lipid profile (cholesterol, LDH and triglycerides) on disease outcome or prognosis?

Methods: This is a prospective observational cohort study consisting of 512 ALS patients, recruited for the TRO19622 (Olesoxime) investigational medicinal product trial. Fasting serum concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured at baseline and during follow-up.

Results: 73% of the participants had hypercholesterolaemia on the screening visit. The prevalence of hypercholesterolaemia decreased with time and was 64% at 18 months follow-up. On univariate analysis total cholesterol, LDL-C and LDL/HDL ratio had a statistically significant effect on survival ($p = 0.015$, 0.003 and 0.027, respectively). On multivariate analysis, however, none of the lipids were found to have a statistically significant effect on survival.

Conclusions: This study does not provide evidence for the lipid profile to be an independent prognostic factor in ALS.

SESSION 8A GENETICS

C67 GENETIC BACKGROUND EFFECTS ON LIFESPAN OF SOD1 MOUSE MODELS OF ALS

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Keywords: modifiers, genetic background, mouse

Background: Dominant SOD1 mutations account for ˜20% of familial forms of ALS. There is wide heterogeneity in age of onset and symptom severity within families carrying the same SOD1 mutation, suggesting that modifier genes significantly impact the disease. Similarly, there is variation in onset and severity in hSOD1Tg mice on genetically heterogeneous backgrounds.

Objectives: The goals of our studies were (1) to test the hypothesis that genetic modifiers can significantly affect the onset or progression of ALS symptoms in G93A mutant SOD1 transgenic mice, (2) to identify QTL loci associated with longevity, and (3) to identify candidate genes affecting the ALS phenotype.

Methods: We developed a range of inbred strains containing the SOD1-G93A mutation with varying lifespans. Long-lived and short-lived strains were used in reciprocal backcrosses for QTL analysis of modifier loci. Reciprocal congenics were used to narrow the QTL interval.

Results: We identified three inbred strains (ALR/LtJ, SJL/J, and NOD/LtSz-*Rag1^{tm1Mom}*) that significantly accelerate disease, and three (C57BL/6J, DBA/2J, and BALB/cByJ) that significantly delay disease. Through reciprocal backcrosses between B6 & ALR and B6 & SJL lines, we have mapped a major QTL on Chr 17 (LOD 11.99) that significantly modifies the lifespan of G93A SOD1 mice.

Additionally, we crossed B6.SOD1-G93A with a B6.NOD-Chr17 congenic covering the entire QTL, and with a more distal B6.SJL-Chr17 congenic line. Lifespan for SOD1 NOD/B6 mice was 149.8 ± 9.0 d, and for SOD1 NOD/NOD mice was 136.4 ± 7.0 d, a statistically significant difference between groups and from the B6.SOD1 line (all $p < 0.001$). The decrease in lifespan is regulated in a dose-specific manner, with one NOD copy resulting in an ~7.1% lifespan reduction, and two NOD copies resulting in an ~15.4% reduction. We have also found that crossing the B6.NOD-Chr17 congenic to the B6.SOD1-G37R line decreases lifespan in a dose-regulated manner. In contrast, crossing to the distal B6.SJL-Chr17 congenic does not affect lifespan. A similar result was found when crossing the short-lived NOD.SOD1-G93A to a distal NOD.B10-Chr17 congenic, which does not increase lifespan. Interestingly, crossing to the distal B6.SJL-Chr17 congenic does decrease age of onset, indicating that the proximal region is critical for both onset and disease duration. We have resequenced the entire Chr 17 region for mutations, and in the proximal region we have identified 10 major genes with non-synonymous coding changes.

Discussion and conclusion: We have demonstrated that genetic background of hSOD1-G93A transgenic mice significantly affects lifespan, and that a region of Chr17 has a major dose-dependent effect on lifespan. The proximal region of this chromosome impacts lifespan for SOD1 mutant mice, while the distal region contains a modifier of onset.

C68 ATXN2 CAG REPEAT EXPANSIONS INCREASE THE RISK FOR CHINESE ALS PATIENTS

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Keywords: ATXN2, polyglutamine, mainland of China

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with unclear etiology. Recently, intermediate CAG repeat expansions in *ATXN2*, the gene responsible for spinocerebellar ataxia type 2 (SCA2), have been identified as a possible genetic risk factor for ALS. In this study, we analyzed the *ATXN2* CAG repeat length in Chinese patients with ALS to evaluate the relationship between the genotype and phenotype. We studied 1,067 patients with ALS and 506 controls from mainland China (excluding Tibet). We collected clinical data and analyzed fluorescent PCR products to assess *ATXN2* CAG repeat length in all of the samples. We observed that intermediate CAG repeat expansions in *ATXN2* (CAG repeat length >30) were associated with ALS ($P = 0.004$). There was no significant difference in clinical characteristics between the groups with and without intermediate CAG repeat expansions in *ATXN2*. Our data indicate that, for ALS patients from mainland China, intermediate CAG repeat expansions in *ATXN2* increase the risk of ALS but have no effect on disease phenotype.

C69   GENOME-WIDE ASSOCIATION ANALYSES IN HAN CHINESE IDENTIFY TWO NEW SUSCEPTIBILITY LOCI FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: GWAS, new susceptibility loci, genetic

To identify susceptibility genes for ALS, we conducted a GWAS in 533 patients with sporadic amyotrophic lateral sclerosis (ALS) and 1,892 controls of Chinese Han. Ninety top SNPs suggested by the current GWAS and six SNPs identified by previous GWA studies were analyzed in an independent cohort of 706 ALS patients and 1,777 controls of Chinese Han. We discovered two new susceptibility loci for ALS at 1q32 (*CAMK1G*, rs6703183, $P_{combined} = 2.92 \times 10^{-8}$, OR = 1.31) and 22p11 (*CABIN1* and *SUSD2*, rs8141797, $P_{combined} = 2.35 \times 10^{-9}$, OR = 1.52). These two loci explain 12.48% of the overall variance of disease risk in the Chinese Han population. We found no association evidence for the previously reported loci in the Chinese Han population, suggesting the genetic heterogeneity of disease susceptibility for ALS between ethnic populations. Our study discovered new genetic susceptibility factors and suggested new biological mechanisms of ALS.

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C70   A GENOME-WIDE ASSOCIATION META-ANALYSIS IDENTIFIES A NOVEL LOCUS AT 17Q11.2 ASSOCIATED WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: genome-wide association study, meta-analysis, heritability

Background: While the genetic architecture of familial amyotrophic lateral sclerosis (ALS) is well characterized, that of the more common sporadic form is poorly understood with only the locus on chromosome 9p21 reliably replicated. At this locus expanded repeats in C9ORF72 gene have been identified as the main causative mutation in familial (23–47%) and sporadic (~5%) ALS cases. As there is evidence for a strong genetic component in sporadic ALS with heritability estimated to be 0.61 in a recent study of 171 ALS twin pairs (1), estimation of additive genetic variance explained by common SNPs can contribute to explain the complex interactions between genes and environment.

Objectives: The major objective of this project was to identify new loci in sporadic ALS. We designed the largest GWAS meta-analysis study to date in ALS combining Italian and international genotype data. A second aim was to estimate the additive genetic variance explained by common SNPs.

Methods: The Italian SLAGEN Consortium collected a novel cohort of 3,959 individuals, while the international ALSGEN Consortium collected GWAS data from 11,611 individuals worldwide (2). After stringent quality controls on individuals and markers, genotype data was analysed for population stratification (EIGENSTRAT). Cleaned genotype data of each study were imputed genome wide (IMPUTE.v2) using the 1,000 Genomes Pilot project (June 2011) as reference panel. Single statistic tables were combined in a final meta-analysis weighting by the inverse of β-coefficients

(METAL). In each study, heritability was estimated using the Genome-wide Complex Trait Analysis software that quantifies the additive genetic variance explained by all SNPs.

Results: We analyzed almost 7 million variants in 13,225 individuals (6,100 cases; 7,125 controls). We confirmed the previously reported association at 9p21.2 (rs3849943, $P = 7.69 \times 10^{-9}$) and identified a novel locus with genome-wide significance at 17q11.2 ($P = 1.11 \times 10^{-8}$) as well as suggestive evidence for a second locus at 18q11.2 ($P = 7.67 \times 10^{-8}$). Functional variants in LD with the lead SNPs were investigated by eQTLs analysis. The contribution of common variation to heritability was ˜12% (95% CI: 0.11–0.13) (data submitted).

Discussion and conclusion: We have identified a novel locus for sporadic ALS risk at 17q11.2, as well as suggestive evidence for a second locus at 18q11.2 and confirmed the association at 9p21. For the first time we have estimated heritability of sporadic ALS. In contrast with twin studies, polygenic variation attributable to common variation does not exceed 0.12. This difference suggests a substantial role for variation not captured by genome wide association studies that can be fulfilled by the detection of rarer variants.

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C71   EXOME SEQUENCING TO IDENTIFY DE NOVO MUTATIONS IN SPORADIC ALS TRIOS

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There have been several recent advances in defining the genetic landscape of ALS. These include discoveries of mutations in *TARDBP*, *FUS/TLS*, *VCP*, *OPTN*, *UBQLN2*, *C9ORF72*, and *PFN1* as new ALS disease genes. Together with mutations in *SOD1*, the causes of over 50% of familial ALS cases have now been elucidated. Despite these extraordinary advances, all together mutations in these genes explain only a small percentage of sporadic cases ($< 10\%$). A possible genetic mechanism for sporadic disease is *de novo* mutation – a mutation that arises spontaneously in the germline of one of the unaffected parents. Indeed, *de novo* mutations have recently emerged as contributors to neurodevelopmental disorders such as autism spectrum disorders, schizophrenia, and mental retardation. We performed the first systematic analysis of ALS trios (ALS patient and both unaffected parents). Because ALS is a late onset disease, trios for which DNA samples are available for patients and their parents are much rarer than for childhood disorders like autism. Nevertheless, we were able to assemble a collection of 50 ALS trios and we performed whole exome sequencing on all 150 individuals ($50 \times 3 = 150$ exomes). To our knowledge, this is the largest collection of ALS trios assembled. We discovered a significant enrichment in *de novo* mutations in genes encoding chromatin regulators, including a *de* novo nonsense mutation in a neuronal chromatin remodeling complex component, SS18L1, and provide evidence that this mutation profoundly affects dendrite outgrowth when expressed in primary neurons. Resequencing this gene in an independent FALS pedigree identified an additional variant, which segregated with disease. Drugs modulating histone acetylation have shown protective effects in ALS mouse models and patient iPSC-derived motor neurons and have undergone phase 2 clinical studies in ALS subjects. Our results now reveal potential genetic connections to ALS as well. These results provide the first systematic analysis of *de novo* mutations in ALS (or any late-onset neurodegenerative disease) and reveal genes encoding chromatin regulators as new candidates for ALS genetic contributors. We propose that the specific genes we identify here, as well as their network of interacting partners (genetic and physical interactions), especially the other components of the SS18L1-containing chromatin remodeling complex are now candidates for evaluation in larger ALS patient cohorts.

C72   USING PUBLIC DATABASES OF GENETIC VARIATION TO TEST THE PATHOGENICITY OF REPORTED ALS MUTATIONS

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Background: Over 400 potential ALS mutations have been reported in the literature but the pathogenicity of most is uncertain.

Objective: To determine whether previously reported ALS mutations occur with too high a frequency among the general population to be disease causing.

Methods: Genomic coordinates and alternate alleles were established for 342 nonsynonymous mutations reported in the Amyotrophic Lateral Sclerosis Online genetics Database (ALSoD). This information was used to screen dbSNP, the 1000 genomes project and the NHLBI Exome Sequencing Project (ESP) for matching records. Where matches were identified, the associated database content was used to evaluate the potential for high penetrance disease causing effects.

Results: One hundred and forty-six of the mutations were identified within at least one of the three reference databases (dbSNP 142; 1000 genomes 28; ESP 56). Given the published lifetime risk of ALS and reported patient carrier frequencies, we determined that 51 mutations occurred too frequently within the 1000 genomes/ESP cohorts to cause ALS with high penetrance. Twenty-two of these mutations related to 'causative' ALS genes (*ANG*, *DAO*, *DCTN1*, *FIG4*, *FUS*, *NEFH*, *OPTN*, *SETX*, *SOD1*, *TARDBP*, and *TAF15*) while 29 related to tentative ALS genes (*CDH13*, *CDH22*, *CRIM1*, *DIAPH3*, *FEZF2*, *GRB14*, *LUM*, *NETO1*, *OMA1*, *SOX5*, *SQSTM1*, and *SYT9*). Notably, our results challenged the pathogenicity of the *DAO*:c.595C>T(p.Arg199Trp) mutation, suggesting that *DAO* may not represent a Mendelian ALS gene. We also found that six mutations mapped to 1000 genomes/ESP records could not be excluded as high penetrance ALS variants at the specified type I error rate ($a = 0.05$). The majority of mutations observed within dbSNP but not within the 1000 genomes/ ESP, had been curated based solely on the observation of carriers among individuals afflicted with ALS and/or other disease phenotypes.

Discussion: Our results cast serious doubt over the pathogenicity of 51 mutations previously associated with ALS. They also highlight the importance of allowing for variable expressivity and the chance inclusion of mutation carriers when using reference populations to evaluate variant pathogenicity.

This has important implications for the conduct of patient resequencing studies.

SESSION 8B TRIALS AND TRIAL DESIGN

C73 THE EFFECT OF TIRASEMTIV ON FUNCTIONAL STATUS IN PATIENTS WITH ALS

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Keywords: skeletal muscle activator, clinical trial, functional benefit

Background: *Tirasemtiv* is a fast skeletal muscle activator that sensitizes the sarcomere to calcium and increases the force of muscle contraction at submaximal simulation frequencies. In both single and multiple dose studies, it has been well tolerated in ALS patients, and dose-dependent improvements in measures of skeletal muscle strength and endurance were suggested.

Objectives: This study was designed to determine the safety, tolerability, and efficacy of *tirasemtiv* administered at up to 500 mg daily on patients with ALS.

Methods: Up to 500 patients with ALS will be enrolled from 73 centers in North America and Europe. Eligibility criteria include a slow vital capacity of greater than 50% of predicted, at least one moderately weak handgrip, and intermediate scores on at least four items in the ALSFRS-R. Patients are randomized to receive either placebo or *tirasemtiv* in a dose escalation protocol up to 500 mg daily given as 250 mg BID for a total of 12 weeks. Prior to randomization, all patients receive open label *tirasemtiv* 125 mg BID for 1 week to ensure that dose is tolerated and to allow adverse events to abate with continued treatment. Patients who tolerate *tirasemtiv* are randomized and begin a flexible 3-week dose escalation to each patient's maximum tolerated daily dose up to 500 mg. Efficacy measures include ALSFRS-R and measures of extremity and respiratory muscle strength and endurance obtained at 4, 8 and 12 weeks during double-blind treatment and at 1 and 4 weeks afterwards. Placebo-treated patients taking *riluzole* receive 50 mg BID; *tirasemtiv*-treated patients taking *riluzole* receive a reduced dose of 50 mg daily to account for the previously described increase in *riluzole* concentration caused by *tirasemtiv*.

Results: All patients will be enrolled in this study by July 2013, with last patient completing double-blind treatment by October 2013.

Discussion and conclusion: This study tests the hypothesis that *tirasemtiv* administered at tolerable doses for 12 weeks can increase skeletal muscle performance resulting in meaningful functional improvements in patients with ALS.

C74 EFFICACY OF ERYTHROPOIETIN IN AMYOTROPHIC LATERAL SCLEROSIS: A MULTICENTRE, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED, PHASE III STUDY (EPOS TRIAL)

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Keywords: erythropoietin, clinical trial, treatment

We designed an independent, multicentre, phase III RCT to assess the efficacy of rhEPO 40.000 UI administered i.v. fortnightly as add on treatment to riluzole 100 mg daily over a 12-month period.

Inclusion criteria were laboratory-supported, probable, or definite ALS according to El Escorial revised criteria, age between 18 and 75 years, sVC 70% or higher, onset 18 months or less. Patients were randomized 1:1 to rhEPO or placebo based on a permuted-block design stratified to ALSFRS-R score (cut-off 33) and onset (spinal or bulbar). Primary outcomes were survival, tracheotomy or more than 23-h NIV for 14 consecutive days and adverse events causing withdrawal. Secondary outcomes were ALSFRS-R decline, sVC decline, and quality of life (ALSAQ-40). Sample size was estimated on 0.8 power (alpha 0.01, two tails).

Analyses were performed by intention-to-treat and per protocol. Twenty-five Italian centers participated in the study. We screened 545 and randomized 208 patients. One patient in

rhEPO and seven patients in placebo were lost at enrolment, therefore 103 patients in rhEPO and 97 patients in placebo entered the study. At baseline, arms (rhEPO and placebo, respectively) were balanced as to gender (55 men and 50 men), mean age (59.4 ± 10; 58.6 ± 10), onset (spinal: 73.8%, 74.2%; bulbar: 26.2%, 25.8%), median ALSFRS-R score (40 (21–48); 39 (20–48)), median sVC (87% (37–110), 86% (23–114)), median ALSAQ40 (98 (43–200); 97 [52–161]), riluzole treatment (97%; 95%). At 12-month follow-up, 38 patients dropped-out and 1 was lost in rhEPO arm, whereas 39 patients dropped-out and 2 were lost in placebo arm. Overall rates for survival (9.7% and 7.2%) and tracheotomy or more than 23-h NIV (14.6% and 15.5%) were non-significantly different between rhEPO and placebo, even after stratification by onset and ALSFRS-R score at baseline. ALSFRS-R decline did not significantly change in rhEPO compared to placebo (−2, 95% CI 0–4), even after stratification by onset and ALSFRS-R score at baseline.

The proportion of adverse events causing drop-out was non-significantly different between rhEPO and placebo arms (16.5% and 8.3%, respectively). Only three events were considered probably associated to rhEPO treatment. At 18-month follow-up, the overall rates for survival (19% and 11.8%) and tracheotomy or more than 23-h NIV (22% and 21.5%) remained non-significantly different between rhEPO and placebo. In conclusion, add-on treatment with i.v. rhEPO 40.000 UI fortnightly did not reduce survival, tracheotomy or NIV rate, neither change the course of ALS over a 12-month period. Treatment was safe and well tolerated.

C75   ADDITIONAL FOLLOW-UP AND BIOMARKER DATA FROM A PHASE II SAFETY AND PRELIMINARY EFFICACY TRIAL OF NP001: A NOVEL IMMUNE REGULATOR FOR SLOWING PROGRESSION OF ALS

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Keywords: clinical trial, therapeutic treatment, neuroinflammation

Background: Abnormal inflammatory macrophages (AIM), systemically and locally in the CNS, are implicated in ALS progression. AIM activation is related to rate of disease progression suggesting that ongoing CNS inflammation may contribute to neuronal death. CNS AIM fuel the ongoing pathogenesis through production of cytokines that attract and drive further AIM migration into the CNS. NP001 is a novel immune regulator that lowers ALS-associated markers of AIM *in vitro* and *in vivo*. In a single-dose phase I trial in ALS patients, NP001 reduced blood AIM inflammatory biomarkers in a dose-dependent manner. NP001 is hypothesized to slow the progression of ALS by modulation of CNS inflammation.

Objectives: To assess the safety and preliminary efficacy of NP001 with additional, recently aquired biomarker and follow-up data, from a phase II trial.

Methods: One hundred and thirty-six patients were enrolled in a randomized, double-blind, placebo-controlled study. Patients met key entry criteria: FVC: >70%, and onset of weakness <3 years. Patients were randomized 1:1:1 to receive 6 months of NP001 1mg/kg/dose, 2 mg/kg/dose or placebo intravenously. Study drug was given as an induction cycle of five consecutive daily doses followed by five monthly cycles of three consecutive daily doses. Patients were seen monthly for 3 months post-dosing to assess durability of effect. The primary and secondary efficacy outcomes were ALSFRS-R slope, with and without matched historical placebo controls, and change from baseline over the 6-month treatment period. Additionally, a post-hoc assessment of non-progressors over the 6-month treatment period was conducted. Safety assessments were conducted throughout the trial. Compared with an earlier presentation, additional follow-up data and the blood inflammatory biomarkers, wrCRP and MCP-1, were assessed before and during the treatment and during a follow-up period without treatment.

Results: One hundred and thirty-six patients were randomized and 115 completed treatment. NP001 was generally safe and well-tolerated. NP001 2mg/kg showed a consistent pattern of slowing progression as assessed by ALSFRS-R slope or change from baseline by 13–21%. Patients with wrCRP greater than median at baseline had greater slowing of disease. NP001 2mg/kg halted disease progression in 27% of patients versus 11% on placebo. Additionally, the majority of responders remained stable during the follow-up period of 3 months without treatment.

Discussion: NP001 2 mg/kg had a modest clinical benefit on slope and unexpectedly halted disease progression in 2.5X as many patients compared to placebo. The absence of progression in most responsive patients, during a 3-month follow-up without treatment, suggests a long-acting effect. Trends in wrCRP support the anti-inflammatory mechanism of NP001. The unprecedented finding that NP001 2 mg/kg halted disease in a subset of patients and the overall benefit-risk support further development.

C76   IDENTIFICATION OF IMPROVED CLINICAL OUTCOMES AND CREATININE-SPARING EFFECT OF DEXPRAMIPEXOLE BASED ON SIGNIFICANT INTER-STUDY DIFFERENCES IN THE PHASE 2 AND PHASE 3 (EMPOWER) CLINICAL TRIALS IN ALS

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Keywords: dexpramipexole, clinical, biomarker

Background: The recent apparent failure of dexpramipexole in the Phase 3 (EMPOWER) trial has heightened questions

about strategies for drug development in amyotrophic lateral sclerosis (ALS). Critically examining the differences in ALS Phase 2 and Phase 3 study designs and conduct may identify factors underlying late-stage drug development failures as well as subpopulations more likely to benefit from particular interventions.

Objective: To determine if significant inter-study differences were present in a post-hoc analysis of the Phase 2 and Phase 3 dexpramipexole trials and whether such differences might account for conflicting trial results and lead to identification of responder subgroups for future clinical trials.

Methods: Baseline characteristics in the Phase 2 and EMPOWER trials were compared to determine whether significant differences were present. Significant differences in baseline characteristics between studies were assessed for their effect on the EMPOWER primary outcome, a Combined Assessment of Function and Survival (CAFS); on the components of CAFS (function, as measured by the ALS-FRS-R change, and survival, as measured by the hazard for mortality), and their potential for identifying responder subgroups.

Results: Significant baseline differences were present in Phase 2/EMPOWER riluzole use (61%/75%, p = 0.002), El Escorial Criteria (EEC) definite ALS participants (46%/32%, p = 0.005), and symptom duration (14.0 months/15.2 months, p = 0.037). Participants with EEC definite ALS had significantly worse CAFS outcomes (468.0 vs. 405.9, p = 0.014) compared with not-definite participants among EMPOWER placebo-treated subjects.

EEC definite ALS (p = 0.013) and symptom duration (p < 0.001), but not riluzole use (p = 0.139), were significant predictors of CAFS outcomes. In the EMPOWER subgroup (n = 147) defined by riluzole use, EEC definite ALS, and short symptom duration (<18 months), participants receiving dexpramipexole (n = 74) versus placebo (n = 73) had improved outcomes on CAFS (416.7/347.7, p = 0.059), ALSFRS-R slopes (−1.24/−1.67, p = 0.006), and mortality (H.R. 0.55, p = 0.080). Treatment with dexpramipexole also significantly reduced the decline from baseline (time averaged difference) in plasma creatinine over 12 months (4.71 μm/l, p < 0.001). The significance of this creatinine-sparing effect increased after adjusting for weight change in dex-treated and placebo-treated participants.

Discussion and conclusion: Significant differences were present in the baseline characteristics of participants enrolled in the Phase 2 and Phase 3 ALS trials of dexpramipexole. In a post-hoc analysis of EMPOWER subgroups selected for these differences, statistically significant benefits of dexpramipexole on ALSFRS-slope and creatinine-sparing and near significant benefits on CAFS and mortality were identified in the subgroup of riluzole-treated, short-symptom duration participants with definite ALS. This subgroup may represent ALS patients with more treatment-responsive disease-related events (faster ALSFRS-R decline/higher mortality) observed over the EMPOWER study period than ALS patients not meeting these criteria. These findings support additional therapeutic trials of dexpramipexole in ALS in a target population enriched for these characteristics, incorporating creatinine as a potential biomarker, and studied for more than 12 months.

C77 FETAL NEURAL STEM CELLS TRANSPLANTATION IN ALS: PRELIMINARY RESULTS OF A PHASE 1 CLINICAL TRIAL

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Keywords: phase 1 clinical trial, foetal neural stem cells, transplantation

Background: There is no cure for ALS. Stem cell therapy represents a promising, perspective therapeutic option. Recently, a meta-analysis of 11 independent studies demonstrated that transplanted neural stem cells (NSCs) can slow both the onset and the progression of clinical signs and prolong survival in ALS mice. Our group has previously documented the integration capacity and potential therapeutic efficacy of hNSC in preclinical studies.

Objectives: We report on an ongoing Phase I trial, aimed at testing safety and feasibility of intraspinal injection of "clinical grade" (produced following the Good Manufacturing Guidelines in a pharmaceutical grade authorized facility) neural stem cells from natural miscarriages into a cohort of 18 ALS patients using a validated surgical apparatus and injection procedures.

Methods: The clinical GMP-status of the NSCs used in this study was granted by the Italian Medicines Agency (AIFA) and the clinical study was approved by the Italian Institute of Health (ISS) as well as by all of the competent ethical committees. An independent Safety Monitoring Board of multidisciplinary experts was also nominated and periodically reviewed and evaluated the accumulated study data.

Until now, six non-ambulatory patients have been recruited whom, following a 3 months observation period, received either unilateral (n = 3) or bilateral NSCs microinjections. (n = 3) into the lumbar spinal cord. For the latter cohort, a maximum of six microinjections was performed. NSCs concentration was 50,000 cells/microliter, for a total of 750,000 cells per injection site. All patients were treated with oral prednisone over 1 month and Tacrolimus in BID oral dosing, over 6 months. Patients are currently being monitored monthly by both standardized clinical and radiological assessment. All the clinical data were recorded in the national database for gene and somatic therapy of the ISS.

Results: No patients manifested severe treatment-related adverse events. The only treatment adverse event was pain noted immediately after surgery, which was confined to the injection sites and the corresponding dermatomes. This was reversible and disappeared after an average 6 days after surgery. Clinical assessments ranging from 6 to 10 months after

transplantation demonstrated no evidence of acceleration of disease progression due to the treatment.

Discussion and conclusion: Our preliminary data confirm the procedural safety of this surgical procedure and show no evidence of immediate or delayed toxicity related to human NSC lines from the brain tissue of single fetuses, established under GMP guidelines and in the absence of ethical concerns, due to the origin of the tissue, derived from fetuses deceased by natural death, and its procurement according to the same international guidelines adopted for organ transplantation. We are now broadening the import of this trial, by testing intraspinal injections into the cervical spinal cord (C5–C6 level), of 12 ambulatory patients.

C78   ANALYSIS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) TREATED WITH AUTOLOGOUS DIFFERENTIATED MESENCHYMAL STEM CELLS: A PHASE I/II AND IIA CLINICAL TRIAL

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Keywords: mesenchymal, stem, cells

Objectives: To evaluate the safety and tolerability of treatment with autologous mesenchymal stem cells differentiated to secrete neurotrophic factors ('MSC-NTF') in ALS patients utilizing intramuscular (IM) and intrathecal (IT) administration.

Background: A previous pilot study from our group at Hadassah has shown the safety of IV/IT administration of unmodified MSC in ALS patients. The neuroprotective effects of MSC-NTF have been demonstrated in various animal models of neurodegenerative diseases, including ALS. In our clinic we are currently conducting the second part of two sequential clinical trials to evaluate the safety and tolerability of autologous MSC-NTF cells in ALS patients.

Methods: In our recently completed Phase I/II clinical study, MSC were isolated from the bone marrow of 12 ALS patients, expanded *ex-vivo* and induced to secrete neurotrophic factors such as GDNF and BDNF using BrainStorm's NurOwn™ technology. These autologous MSC-NTF cells were transplanted by IM (at 24 sites: 2×10^5 cells per site) or IT (1×10^6 cells/kg) injections to patients with early (ALSFRS score of > 30; n = 6) or advanced ALS (ALSFRS: 15–30; n = 6), respectively. All patients were followed up clinically on a monthly basis for a pre-treatment period of 3 months and for 6 months post-transplantation. Respiratory function tests, 3D-MRI of the muscles and compound muscle action potential amplitudes at three sites were used as additional surrogate markers of disease activity.

Results: During the six-month follow-up of the 12 transplanted patients, no serious treatment-related adverse events were observed, indicating short-term treatment safety. The clinical follow-up revealed a change in the rate of clinical progression (ALSFRS) and respiratory function (FVC) in favor of the IT-treated patients during the 6 months following treatment, as compared to the 3 months preceding treatment.

Conclusions: This first pilot trial in 12 patients with ALS showed that intrathecal or intramuscular injection of MSC-NTF is safe and revealed some indications of clinical beneficial effects. In the second part of the ongoing Phase IIa dose-escalating trial in our Center, 12 additional ALS patients are currently receiving combined IM and IT treatment with escalating doses of MSC-NTF cells, up to twice those administered in the Phase I/II trial. Initial observations from the first treated patients do not show any serious adverse events up to date. More detailed and updated data from this trial will be presented.

SESSION 9A GLIAL BIOLOGY AND PATHOLOGY

C79   OLIGODENDROCYTES: FROM BIOLOGY TO DISEASE

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Keywords: epigenetics, glia, chromatin

Recent advances have suggested an important role of myelin in plasticity and axonal survival. Myelin is formed by mature oligodendrocytes, which derive from oligodendrocyte progenitors. This presentation will address the progression from progenitors to myelinating cells, by reviewing the integration of extracellular factors with transcriptional networks and epigenetic modifiers. Epigenetic regulation of oligodendrocyte differentiation includes the study of histone-specific enzymatic activities, DNA methylation, and microRNAs. Epigenetic changes reflect the effect of environmental components on gene expression and as such, they play an important role in development and pathology.

C80   OLIGODENDROCYTES FROM THE ALS MOUSE MODEL AND ALS PATIENTS ARE TOXIC TO MOTOR NEURONS *IN VITRO*

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Keywords: motor neurons, oligodendrocytes, co-culture

Background: In the past few years, it has become clear that non-neuronal cells are major players in dictating disease progression rate in ALS (1). It has been very recently reported that oligodendrocytes are pivotal in providing metabolic support to MN apart from regulating ion conductance through the axon (2).

Two studies so far have reported that the spinal cords of ALS patients as well as the mouse model are affected by morphological changes in grey matter oligodendrocytes that ultimately die during disease progression (2, 3). New oligodendrocyte precursor (NG2+) cells proliferate, but fail in reaching full maturation, thus leaving MN un-myelinated and deprived of metabolic support.

In this context, we have successfully developed *in vitro* systems to study oligodendrocyte differentiation and toxicity to MN from both the ALS mouse model and human samples.

Methods: Primary mouse NG2 + cells were isolated from the cortex of neonate (P2) SOD1^{G93A} and wild-type mice and either used 24h after isolation for co-culture with HB9:GFP+ MN or differentiated for 7 days into MBP+ oligodendrocytes and then co-cultured with HB9:GFP+ MN.

Human skin fibroblasts were converted to induced pluripotent stem (iPS) cells and these were then differentiated to tripotent neural progenitor cells (NPCs). NPCs were subsequently differentiated into MBP+/GalC+ oligodendrocytes by supplementing the medium with PDGF-AA and IGF-1 at different concentrations for one month.

Results: Data analysis showed that MBP+ oligodendrocytes from SOD1^{G93A} mice, but not NG2 + cells, are toxic to wild-type mouse MN after 6 days in co-culture, resulting in 40% decrease in MN survival and 50% decrease in axonal length.

Strikingly, human MBP+/GalC+ oligodendrocytes from ALS patients cause a dramatic decrease in HB9:GFP+ MN survival with only 50% of the cells surviving after 48h from plating and only 20% after 72h. Moreover, one of the most compelling characteristics of this co-culture system is the axonal phenotype displayed by MN plated onto ALS samples with marked axonal beading or retraction.

Finally, we developed a reliable tool to visualize mature oligodendrocytes *in vivo* using an AAV9 vector expressing green fluorescent protein (GFP) under the myelin basic protein (MBP) promoter in order to monitor oligodendrocyte fate during disease progression.

Conclusions: Our data confirm and build upon the present knowledge that oligodendrocyte cells are dysfunctional in ALS. Moreover, this study provides the first *in vitro* model to investigate the toxic properties of human ALS oligodendrocytes.

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C81   ALTERED ASTROCYTIC EXPRESSION OF TDP-43 DOES NOT INFLUENCE MOTOR NEURON SURVIVAL

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Keywords: TDP-43, astrocytes, non-cell autonomous

Background: Recent studies have highlighted a role for glial cells in ALS pathogenesis. Nevertheless, the majority of studies implicating glia have focused on transgenic mouse models of mutant superoxide dismutase (SOD1) expression. Recently, mutations in tar DNA-binding protein 43 (TDP-43) have been linked to ALS. It is still unknown whether TDP-43 mutations cause ALS through a gain or loss of function mechanism, however, studies have indicated that regulation of TDP-43 expression is critical for maintaining normal function. Rodents with altered levels of TDP-43 expression develop an array of ALS phenotypes, but the contribution of astrocytes to TDP-43-linked ALS has not been investigated in these models.

Objectives: To determine whether astrocytes either overexpressing mutant TDP-43 or lacking TDP-43 can damage wild-type (WT) motor neurons (MNs) either *in vitro* in a co-culture system or *in vivo* after transplantation into the spinal cord of WT rats.

Methods: Glial-restricted precursors (GRPs) were isolated from mice overexpressing TDP-43[A315T] or from Ubiquitin-ER-Cre;TDP-43[flox/-] mice to use as a model for TDP-43 knockout. WT littermate and SOD1[G93A] mice were used as controls. The GRPs were differentiated to astrocytes *in vitro* or transplanted to the spinal cord of WT rats for *in vivo* astrocyte differentiation. The effects of the astrocytes on WT MN survival were determined *in vitro* using a co-culture system or *in vivo* by examining host MNs at 3 months post-transplantation.

Results: Astrocytes overexpressing TDP-43 or lacking TDP-43 were cultured with WT MNs *in vitro*. Decreased MN survival was observed with SOD1[G93A] astrocyte co-culture, but no change in MN survival was noted between WT astrocytes and astrocytes with alterations in TDP-43 levels. To examine the effects of TDP-43 alterations in astrocytes on WT MNs *in vivo*, WT, TDP-43[A315T], Ubi-ER-Cre;TDP-43[flox/-] and SOD1[G93A] GRPs were transplanted to the cervical spinal cord of WT rats, where they differentiate to astrocytes. To induce TDP-43 knockout in the transplanted Ubi-ER-Cre;TDP-43[flox/-] cells *in vivo*, rats were injected with tamoxifen. Rats receiving SOD1[G93A] astrocytes showed a marked decline in forelimb grip strength over time which correlated with a loss of cervical MNs. However, no MN loss or behavioral deficits were detected after transplantation of WT or TDP-43[A315T] astrocytes or after knockout of TDP-43 in engrafted Ubi-ER-Cre;TDP-43[flox/-] astrocytes *in vivo*.

Conclusions: Our data show that altering the levels of TDP-43 expression by either knocking out TDP-43 or overexpressing an ALS-linked TDP-43 mutant does not cause MN degeneration either *in vitro* or *in vivo*, implying that astrocytes may not be involved in human ALS caused by TDP-43 mutations. Our study highlights the probable heterogeneity in ALS disease mechanisms and underscores the importance of evaluating subsets of ALS patients for differences in disease pathways as novel genetic contributors are uncovered.

C82   MUTANT TDP-43 TRIGGERS ASTROCYTIC ACTIVATION AND IMPAIRED GLUTAMATE TRANSPORT IN PRIMARY CULTURES DERIVED FROM TDP-43A315T MICE

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Keywords: astrocytes, glutamate, TDP-43

Background: Cytoplasmic accumulation of pathological TDP-43 occurs in motor neurons and glial cells in ALS. The presence of TDP-43 pathology in astrocytes and astrocytic activation in ALS suggests non-cell autonomous effects of mutant or misfolded TDP-43 on motor neuron degeneration, as previously described for mutant SOD1-mediated ALS. Astrocytes can mediate injury to motor neurons by secretion of proinflammatory factors, reduced neurotrophic factor support and glutamate-mediated excitotoxicity. To address whether mutant TDP-43 action within astrocytes is harmful to motor neurons, we established and characterised primary astrocyte cultures expressing ALS-linked mutant TDP-43.

Objectives: To generate and characterise cell-autonomous effects of mutant TDP-43 on astrocytes and non-cell autonomous effects on motor neurons using primary cultures derived from transgenic TDP-43[A315T] mice.

Methods: Primary astrocyte and motor neuron cultures were established from cortices and spinal cords of neonatal C57BL/6 or transgenic TDP-43[A315T] mice. Astrocytes were cultured for 2 weeks and TDP-43 pathology, solubility and subcellular distribution was analysed using Western blotting and immunocytochemistry. Astrocyte morphology, proliferation and cytoskeleton were analysed using immunocytochemistry. Functional activity of astrocytes was examined using [³H]D-aspartate uptake assay. Astrocyte and motor neuron co-cultures were established to test non-cell autonomous effects of mutant TDP-43.

Results: Western blotting analysis showed similar levels of endogenous and mutant TDP-43 accumulation in both soluble and insoluble fractions of astrocyte cultures and no evidence for abnormal phosphorylation, ubiquitination or truncation of mutant TDP-43. Mutant TDP-43 was also predominantly localised to the nucleus in transgenic TDP-43[A315T] astrocyte cultures. However, GFAP expression was significantly increased and [³H] D-aspartate uptake activity was significantly diminished in transgenic TDP-43[A315T] astrocytes, compared to WT cultures. Astrocyte activation was not due to changes in microfilaments determined by F-/G-actin staining in TDP-43[A315T] astrocytes.

Discussion and conclusion: Our findings demonstrate activation and impaired glutamate uptake in astrocytes derived from transgenic TDP-43[A315T] mice, consistent with cell-autonomous effects of mutant TDP-43 on astrocytes. The toxic potential of TDP-43[A315T] astrocytes to motor neurons is currently under investigation.

C83   HUMAN SPORADIC ALS AND RODENT FAMILIAL ALS PRIMARY ASTROCYTES ARE SELECTIVELY TOXIC TO SPINAL MOTOR NEURONS THROUGH THE SAME DEATH PATHWAY

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Keywords: non-cell autonomous toxicity, astrocytes, motor neuron death

Although 90% of ALS cases are sporadic, the lion's share of attention in ALS research has been paid, so far, to the

remaining 10% that are inherited and present mutations in different genes. Rodents expressing human mutations for superoxide dismutase-1 (SOD1), the most commonly mutated gene in ALS families, develop a paralytic disorder that emulates clinical and pathological hallmarks of ALS. The cellular mechanisms leading to motor neuron death remain, however, largely unknown. The objectives of our research are to decipher the cellular and molecular mechanisms leading to motor neuron death in ALS and to identify new valuable therapeutic targets for the treatment of this debilitating disease.

Previously, we have shown *in vitro* that primary astrocytes produced from mutant SOD1 rodent models of ALS are selectively killing motor neurons through the release of soluble toxic factors (Nagai *et al* 2007). Recently, we successfully produced primary astrocytes from sporadic ALS (n = 6) and age-matched control (healthy (n = 7), chronic obstructive pulmonary disorders (n = 6) and Alzheimer's (n = 3)) patient brain tissues. Here, we show that human sporadic ALS and control astrocytes exhibit a series of classical astrocyte markers, and a similar morphology and index of viability. However, human sporadic ALS astrocytes are selectively toxic to co-cultured mouse or human stem cell-derived motor neurons compared to astrocytes produced from control patients. Our data also suggest that both rodent and human ALS astrocytes recruit the same caspase-independent, but Bax/JNK3-dependent death pathway in motor neurons with features of necrosis. Furthermore, we provide evidence that this form of programmed necrosis relies on the receptor-interacting protein 1 RIP1 and mixed lineage kinase domain-like protein MLKL suggesting that necroptosis is the motor of cell death in both familial and sporadic ALS models.

Taken together, our findings suggest that astrocytes may also have a crucial role in motor neuron death in human ALS and, more importantly, that diseased astrocyte toxicity may be relevant to both the familial and the sporadic forms of ALS. JNK3, which is predominantly expressed in the central nervous system, is a promising molecular target for ALS therapy and is currently tested in a preclinical study in SOD1^{G93A} mutant mice.

SESSION 9B DISEASE PROGRESSION

C84   ALS/MND AS A DISEASE SPECTRUM: TIME TO LEAVE THE LUMPERS BEHIND?

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Keywords: frontotemporal dementia, motor neuron phenotype, genetics

While ALS/MND (ALS) has been traditionally described as being a single disease process, it is clear that this has never really been the case. Hyperendemic foci such as that described amongst the Western Pacific and Kii Peninsula and familial clustering long ago supported the concept that as a clinical disease entity, hence ALS must represent only the final expression of a broad range of pathological processes. Clinicians accept that any given individual will manifest with a largely predictable disease course, but that there is little consistency amongst individuals. We accept that ALS can be highly restricted and present with pure lower motor neuron, pure upper motor neuron, bulbar or neurocognitive deficits, and that these presentations are prognostically relevant. We further accept that there are individuals who will have a rapidly progressive disease course with survival of less than a year (a 'malignant' variant), a more typical course with survival of 3–5 years, or a markedly prolonged course with survival of greater than 10 years. The suggestion that young males with hand onset symptoms will be more likely find themselves in this latter, more benign category, suggests that the use of such clinical differentiators has important pathological correlates.

This thesis is further supported by the presence of frontotemporal dysfunction in a significant proportion of patients in whom disruption of higher cognitive networks finds expression in a broad array of neurocognitive deficits ranging from a frontotemporal dementia, a cognitive or behavior syndrome, or deficits in Theory of Mind (and thus reflective of more mesial frontal involvement). The critical point here is that none of this is reflective of pure motor involvement, but rather other, presumably equally discrete, cellular pathology.

The era of modern cellular and molecular biology further illustrates that ALS cannot be considered as a single disease process. The current genetic taxonomy of ALS highlights two broad pathways of disease process that include fundamental alterations in the processing of RNA, or in protein folding and degradation. Even within these two broad categories, the molecular biology is complex and again suggestive of discrete biologies, often associated with specific alterations in the expression of disease process as is illustrated by the spectrum of clinical manifestations even within a single mutated gene (SOD1).

In the end, the concept that ALS should be considered as a spectrum of biological processes of limited phenotypic expression, as we first proposed over 15 years ago still holds true and is now an inescapable conclusion. The next challenge will be to translate this knowledge into meaningful clinical trial design.

C85   BEING PRO-ACTIVE - WHAT A CLINICAL TRIALS DATABASE CAN REVEAL ABOUT ALS

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Keywords: clinicial trials, patient data, disease progression

Large datasets are critical for identifying statistically significant and biologically relevant observations, especially in rare disease like ALS. The Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform provides an unprecedented opportunity to increase our understanding of the ALS patient population. The database consists of over 8500 ALS patients who participated in 17 clinical trials funded by industry and the non-profit and government sectors.

Data include demographic, family history, vital sign, clinical assessment, lab data, medication, and survival information. The PRO-ACT database was made open-access to researchers worldwide at the 2012 International Symposium on ALS/MND, and in the first 4 months the data have been downloaded by more than 125 different researchers from 18 different countries.

Analysis of the PRO-ACT data has revealed several novel and important findings which will be presented. The first of these is the identification of several novel baseline (ie prognostic) variables that significantly correlate with ALSFRS slope in a multivariate analysis (controlling for time from onset, age, gender, and baseline functional measures). The second is the demonstration of unexpected differences between active and placebo groups, in spite of well-matched demographics, riluzole use, and other enrollment criteria. In addition, several initial stratification models will also be presented.

Given the open-access nature of the PRO-ACT data, in addition to an internal analysis we undertook an innovative crowdsourcing initiative to shed light on the difficult challenge of ALS prognosis. The DREAM- Phil Bowen ALS Prediction Prize4Life was launched to incentivize the development of improved methods to accurately predict future change in ALSFRS at the individual patient level. This program brought in over 1000 solvers from around the world and led to the development of several valuable algorithms to predict the progression of ALS, with potential to aid both clinicians and future ALS clinical trials.

The challenge also led to the identification of new features predictive of ALSFRS progression that have now been verified using the full PRO-ACT dataset as well as in a recent large independent Phase III dataset, and these data will be presented.

These early results demonstrate the value of large datasets for developing a better understanding of ALS natural history, prognostic factors, and disease variables. More sophisticated

and targeted analyses will continue to reveal new insights into this disease, which has for so long defied our understanding.

C86 ISOMETRIC MUSCLE TESTING USING HAND-HELD DYNAMOMETRY (HHD) IN A MULTICENTER ALS TRIAL

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Keywords: outcome measures, strength, clinical trial

Background: The decline in muscle strength is an important determinant of disability in ALS. Strength has been employed as an outcome measure in clinical trials for many years, with strength measured both qualitatively (MRC grading) or quantitatively with strain gauges. We have developed a method of assessing strength of multiple muscles using a hand-held dynamometer (HHD), and have assessed strength using HHD in the recently completed trial of ceftriaxone in ALS.

Objectives: To study changes in muscle strength assessed using HHD of ALS patients enrolled in the ceftriaxone study over the course of 1 year, and to compare HHD with other outcome measures employed in this trial.

Methods: Isometric muscle strength was measured over time in nine upper and lower extremity muscles bilaterally, for 513 subjects enrolled. All measurements were obtained with the subjects seated in a chair, with standard positions defined for each muscle for both evaluator and subject. Formal training of evaluators was conducted, and a criterion value of test–retest reliability on four normal subjects was required prior to patient testing. All muscles were tested to "break", that is, the force required to induce movement in the muscle studied was recorded as the maximum force. Subjects were studied every 3 months for the duration of the study, but 1 year results are reported here.

Results: Data were evaluated using absolute force measurements, and Z scores were constructed for each value using both data from 240 normal subjects and from the screening visits for ALS subjects participating in the study. For each muscle, slope of decline over 1 year was determined, and the coefficient of variation (CoV) for rate of change calculated. The same measures were obtained for muscles combined as megascores from upper and lower extremities as well as a total body megascore. CoV for individual muscles ranged from -1.01 to -1.61. Megascore CoVs were -0.96 for upper extremities, -0.92 for lower extremities, and -0.85 for all muscles combined. Decline in muscle strength was highly correlated within a limb, and within body segment. Arm and leg muscles were less well correlated.

Discussion and conclusion: With formal training and validation, HHD provides a highly reliable method of measuring muscle strength in ALS patients. HHD is highly correlated with both ALSFRS-R and VC, and is comparable with these measures with respect to CoV for rate of change. Combinations of multiple muscles using megascores reduces CoV, and the high correlation of muscle strength within a limb allows

for the use of a more restricted number of muscles than the 18 separate muscles evaluated here.

C87 BULBAR ALS: PREDICTING SURVIVAL FROM PHYSIOLOGICAL MEASURES OF SPEECH

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Keywords: Bulbar, speech subsystems, survival

Background: Bulbar ALS is a fast progressing and perhaps the most devastating form of ALS. Only 25% of patients initially present with bulbar signs and symptoms, yet 75% of patients show bulbar disease as ALS progresses (1). The bulbar system is anatomically diverse and composed of the laryngeal, velopharyngeal, facial and oral articulatory subsystems. These subsystems are differentially impaired with previous reports suggesting that the tongue might be the earliest and most severely involved bulbar component (2). Disease survival has been estimated in ALS based on various factors. The prognostic value of bulbar decline for predicting survival has not been addressed with the exception of tongue weakness (3).

Objectives: The primary aim of this study was to identify the measures of bulbar function that affect survival across all speech subsystems.

Methods: One hundred and forty-six participants diagnosed with ALS (Mean age = 59.02 years, SD = 10.3) were recorded every 3 months for the average duration of 22.11 months (SD = 16.74). Thirty-four individuals presented with bulbar onset ALS, the remaining individuals presented with the spinal onset. The average ALSFRS score at the first session was 36.84 (SD = 6.56). The protocol and measurements are described in detail elsewhere (4). Survival was estimated as the number of months from symptom onset to death, and was available for 60% of participants. Patients those remained alive were censored at the time of the last recording session. The prognostic value of each predictor variable was estimated using the Cox proportional hazard analyses.

Results: The results showed that bulbar onset and %FVC were significant predictors of survival. Speech measures, including intelligibility and speaking rate, as well as indicators of velopharyngeal incompetence (eg nasal flow during syllable /pa/) and tongue dysfunction were able to predict survival.

Discussion and conclusion: Objective physiological measurements of bulbar dysfunction were able to predict survival in ALS. They are important to monitor at the time of diagnosis and as disease progresses. The ultimate goal of this work is to predict, based on the instrumental bulbar assessments,

the course of bulbar disease progression for individuals and cohorts in clinical trials.

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C88   THE SOLEUS H-REFLEX DELINEATES UPPER MOTOR NEURONE PATHOPHYSIOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: electrophysiology, biomarker, upper motor neuron

Background: The H-reflex has been used extensively to interrogate mechanisms of UMN dysfunction in neurological disorders, but its value in patients with ALS has not been established.

Objectives: The present study was undertaken to characterise the pathophysiological changes in the soleus H-reflex in amyotrophic lateral sclerosis (ALS), to relate these changes to the clinical phenotype, and to evaluate its potential use as an objective measure of upper motor neuron (UMN) dysfunction.

Methods: Recruitment curves of bilateral soleus H-reflex and M-wave were recorded with an automated system (QTRAC) in 28 patients with ALS and 15 age-matched control subjects. Analysed parameters included $H_{max}:M_{max}$ ratio (H_{max}/M_{max}), and the minimum intensity required to produce a H-reflex and M-wave (H_{thresh} and M_{thresh}, respectively). The slope angles of the linear ascending portion of the H and M recruitment curves (H_θ and M_θ, respectively) were calculated and H_θ/M_θ was derived. In ALS patients, clinical UMN dysfunction was assessed with a quantitative scale (UMN Score, UMNS) grading hyperreflexia at the knee and ankle, and the presence of the Babinski response and ankle clonus. Additional clinical assessments included grading of thigh, leg and foot wasting (Wasting Score, WS), and a composite lower limb muscle strength score graded using the Medical Research Council grading system. The UMNS was incorporated into separate, preplanned, multiple linear regression models for H_{max}/M_{max} and H_θ/M_θ.

Results: H reflexes were identified in 92% of limbs of ALS patients, with absent H-reflexes only associated with marked reduction of M_{max} (0.11–1.30mV). Significant differences in H-reflex parameters were noted in the ALS group with reduced M_{max} ($p = 0.001$), H_{max} ($p < 0.05$), and M_θ ($p < 0.05$), and increased H_θ/M_θ ($p < 0.05$). Unexpectedly, H_{max}/M_{max} and H_θ were similar between groups. Both H_{max}/M_{max} and H_θ/M_θ were strongly predicted by clinical UMN dysfunction ($p < 0.001$), but H_{max}/M_{max} was also noted to decrease significantly with age ($p = 0.001$). Further analysis identified that H_{max}/M_{max} in ALS patients was predicted by the position of the H-recruitment curve relative to the M-recruitment curve.

Discussion: Parameters derived from the soleus H-reflex are closely linked with UMN dysfunction in ALS. Based on analysis of the relationships between H_θ, M_θ and other H-reflex parameters, it is hypothesised that alterations in lower motor neurone excitability following UMN injury may contribute to the changes in the H-reflex pathway in ALS. In addition, H_{max}/M_{max} may be more susceptible to the influence of age, and collision of the H-reflex with antidromically conducted impulses arising from directly stimulated motor axons in patients with ALS, and thus may not be considered the optimum measurement.

Conclusions: The present study demonstrated that measurement of the H-reflex provided insights into the pathophysiology of ALS and established the H-reflex as a robust objective measure of UMN dysfunction.

SESSION 10A PROTEIN PROCESSING AND DEGRADATION

C89   MECHANISMS OF PRION-INDUCED TOXICITY

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Keywords: prions, mice, toxicity

Prions cause neurodegeneration *in vivo*, yet prion-infected cultured cells are asymptomatic. This has hampered mechanistic studies of prion-induced neurodegeneration. We have found that prion-infected cultured organotypic cerebellar slices (COCS) experienced progressive spongiform neurodegeneration closely reproducing prion disease, with three different prion strains giving rise to three distinct patterns of prion protein deposition. Neurodegeneration did not occur when the Prnp gene was genetically removed from neurons, and a comprehensive pharmacological screen indicated that was abrogated by compounds known to antagonize prion replication. Prion infection of COCS led to enhanced fodrin cleavage, suggesting the involvement of calpains or caspases in pathogenesis. Accordingly, neurotoxicity and fodrin cleavage were prevented by calpain inhibitors, but not by caspase inhibitors, whereas prion replication proceeded unimpeded. Hence, calpain inhibition can uncouple prion replication from its neurotoxic sequelae. These data validate COCS as a powerful model system that faithfully reproduces most morphological hallmarks of prion infections. The exquisite accessibility of COCS to pharmacological manipulations was instrumental in recognizing the role of calpains in neurotoxicity, and significantly extends the collection of tools necessary for rigorously dissecting prion pathogenesis.

The cellular prion protein PrPC consists of a globular domain (GD) hinged to a long N-proximal flexible tail (FT). We found rapid neurodegeneration in mice and in COCS exposed to holoantibodies, monovalent F(ab)1 fragments, or single-chain miniantibodies targeting the α1 and α3 helices of the GD. Degeneration was prevented by interstitial deletions within the FT and by treatment with various FT ligands, indicating that GD ligand toxicity was executed by the FT. Antibodies to the FT also prolonged the life of mice expressing a toxic PrPC mutant (PrP 94–134). These data uncover an essential role for the FT in two models of prion-related toxicity, and indicate that the FT triggers shared downstream effectors of neurodegeneration.

C90   *IN VIVO* PROPAGATION OF HUMAN WILD-TYPE SOD1 MISFOLDING IN A TRANSGENIC MOUSE MODEL

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Keywords: superoxide dismutase 1, propagated protein misfolding, mouse model

Background: One of the mechanisms by which a mutant or wild-type (Wt) protein can dominate pathogenesis of phenotypically diverse diseases is by propagated protein misfolding, such as that underpinning the prion diseases, which has been increasingly implicated in other neurodegenerative and systemic disorders (1, 2). A role for propagated protein misfolding in ALS is supported by the prion-like spatiotemporal progression of disease through the neuroaxis (3). Prion-like activity has been described for the cell-to-cell transmission of misfolding of mutant SOD1 (4), and we have reported that mutant SOD1 can confer its misfold on HuWtSOD1 in a species-dependent manner; however, mutation of misfolded SOD1 at residue tryptophan-32 (W32) abolishes this process (5). Here we report that propagated SOD1 misfolding can occur in an *in vivo* mouse model system.

Methods: Purified mutant SOD1 proteins G127X and G127X/W32S were injected into the left and right brain hemispheres (cortex area-primary motor cortex region), respectively, of human wild-type (HuWt) SOD1 transgenic mice and non-transgenic littermates. Tissue from the injection site was then analyzed by immunohistochemistry at 7 days, 1 and 2 months post-injection in order to detect if misfolded HuWt SOD1 is present, indicative of a propagated protein misfolding event induced by truncated SOD1 mutant G127X.

Results: Staining of misfolded HuWtSOD1 in mouse spinal cord was detected 1 and 2 months post-injection with purified G127X-SOD1 protein in cells surrounding the injection site using our SOD1 disease-specific antibodies. In contrast, no misfolded SOD1 staining was observed away from the injection site in tissue injected with G127X/W32S protein at the corresponding time-points. Likewise, misfolded HuWt-SOD1 was not observed away from the injection site in non-transgenic mice as no endogenous HuWtSOD1 is available as a substrate for propagated protein misfolding.

Conclusion: Mutant misfolded SOD1 can impart its misfold on HuWtSOD1 in an *in vivo* mouse model. This process is species-specific as human G127X SOD1 does not propagate misfolding of endogenous mouse SOD1 and is restricted to the availability of residue W32, as previously described *in vitro*. Our results support the notion that cell-to-cell spread of SOD1 misfolding contributes to the systematic spread ALS pathology through the neuroaxis.

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C91   OVEREXPRESSION OF HUMAN WILD-TYPE SOD1 HASTENS DISEASE ONSET AND INDUCES EARLIER PRESENCE OF MUTANT SOD1 AGGREGATES IN A MOUSE MODEL OF ALS

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Background: Mutations in superoxide dismutase-1 (SOD1) cause familial amyotrophic lateral sclerosis (ALS). Since SOD1 mutations are mostly dominantly inherited, SOD1 mutant and wild-type SOD1 ($SOD1^{WT}$) are co-expressed in the patients. When both proteins are co-expressed in double transgenic mice, both mutant and $SOD1^{WT}$ form aggregates, and $SOD1^{WT}$ exacerbates disease course of mutant SOD1 mice. However, direct *in vivo* molecular behaviors of both proteins remain elusive.

Objectives: The aim of the present study was to determine direct molecular behaviors of both $SOD1^{WT}$ and SOD1 mutant during disease course of double transgenic mice.

Methods: To allow distinction between $SOD1^{WT}$ and SOD1 mutant in double transgenic system, we selected $SOD1^{G127insTGGG}$ ($SOD1^{G127X}$) mice. Unique sequences of $SOD1^{G127X}$ enable us separate both proteins even on conventional immunoblotting using mutually exclusive antibodies: one directed against the five non-native amino acids following Gly127 in $SOD1^{G127X}$, the other directed against the C-terminal 23 amino acids of $SOD1^{WT}$ truncated in the mutant.

To generate double transgenic mice, homozygous $SOD1^{G127X}$ mice were crossed with hemizygous $SOD1^{WT}$ mice (line N1029). Disease onset was defined as the time when mice reached peak body weight. The end-point was defined as the age at which a mouse was unable to right itself within 5s after being pushed onto its side. Disease duration was determined as the period from disease onset to the end-point.

Spinal cords were harvested at three different stages of disease including presymptomatic, symptomatic, and terminal stage. For analysis of SOD1 aggregates, detergent-insoluble fractions were extracted from spinal cords, and the fractions were investigated using immunoblotting.

Results: The disease onset in double transgenic mice was significantly earlier than in $SOD1^{G127X}$ mice by 49% from 377 ± 26 days to 192 ± 3.8 days. The lifespan was also markedly shortened from 422 ± 33 days to 238 ± 5.6 days, representing a decrease of 43%. There was no difference in the disease duration. The $SOD1^{G127X}$ aggregates in double transgenic mice were significantly increased even at a presymptomatic stage. These aggregates were accumulated in a disease stage-dependent manner. $SOD1^{WT}$ also formed aggregates in double transgenic mice, but they were found after the onset of symptoms. Insoluble murine SOD1 was not detected in double transgenic mice in all tested disease stages.

Conclusion: $SOD1^{WT}$ aggregated in the double transgenic mice and induced earlier presence of $SOD1^{G127X}$ aggregates. This resulted in acceleration of the disease onset.

C92   PROTEIN STABILITY AND NEURODEGENERATIVE DISEASE

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Background: Protein stability and aggregation are an often recurring theme in the aetiology of neural disease. A synergistic interaction between mutant SOD1 aggregation propensity and instability strongly influences patient survival time after disease onset. In the final stages of SOD1 maturation, the copper chaperone for SOD1 (CCS) transfers copper and a disulphide bond to SOD1 leaving a stable and active enzyme. Complex formation between hCCS and SOD1 is mediated by the zinc-binding SOD1-like hCCSD2. Without effective post-translational modifications (PTMs), nascent SOD1 is structurally compromised. PTMs also play a large part in TDP-43 stability. Increased *in vivo* half-life is a common feature mutant and modified wild-type TDP-43. This characteristic shows an inverse correlation with patient age at onset of disease symptoms, however, its molecular cause is not known.

Objective: Determine how missense mutations and drug molecules affect the structure and integrity of TDP 43, SOD1, hCCS and the heterodimeric SOD1-hCCS complex.

Methods and Results: Using X-ray crystallography, we have discovered a novel binding site for catecholamine neurotransmitters in the SOD1 β-barrel at loop 2. We found cisplatin and bismaleimidoethane increase mutant SOD1 stability and completely inhibit aggregation, respectively. Using small angle X-ray scattering with online SEC, we have been able to describe wild-type hCCS, an R163W mutant hCCS and also the hCCS-SOD1 complex. In this last case, inclusion of ALS SOD1 mutations appears to maintain the overall conformation of the complex. However, we find that the disease-related R163W hCCS mutation reduces zinc binding by hCCSD2 and causes normally stable dimeric hCCS to monomerise. This mutant protein is also susceptible to thermal unfolding and is aggregation prone. Like Zn-apo wild-type, R163W hCCS cannot form the critical heterodimeric complex with SOD1. By contrast, we find that ALS and FTLD mutations in the TDP-43 nucleic acid binding domains increase the protein's stability. This is maintained and elevated when DNA bound.

Discussion and conclusion: Amelioration of instability and aggregation is considered the target for SOD1 directed

therapeutics. Cisplatin and bismaleimidoethane have previously been shown to inhibit aggregation and stabilise SOD1, respectively. We have shown that both characteristics are rescued by these compounds in a manner which is equal but opposite to the detrimental effects of SOD1 mutations. Our work with R163W hCCS indicates that it recapitulates the molecular phenotype of ALS SOD1 mutants; dimer instability and monomerisation leading to aggregation. These observations propose the question: Could SOD1 ALS be a result of ineffective interactions with hCCS? In contrast, TDP-43 displays a gain in stability conferred by ALS and FTLD-U mutations which do not cause unusual inter-domain interactions or unfolding, but may reduce protein turnover by inhibiting degradation.

C93 STUDYING AGGREGATION AND DISTRIBUTION OF TDP-43 IN MAMMALIAN CELLS USING BIARSENICAL LABELLING

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Keywords: TDP-43, protein aggregation, biarsenical labelling

Background: The aggregation and deposition of transactivation response DNA-binding protein 43 (TDP-43) in motor neurons is a key pathological feature in nearly all amyotrophic lateral sclerosis (ALS) cases (1). Despite this, relatively little is known about the mechanism of TDP-43 aggregate formation. To date, a detailed analysis of the dynamic processes leading to such aggregate deposits in live cells has not been feasible.

Objectives: We used the small tetracysteine tag (TC-tag) and corresponding biarsenical dyes (FlAsH and ReAsH), along with intermolecular FRET-based microscopy to analyse the protein dynamics of TDP-43 inside live mammalian cells.

Methods: Human neuroblastoma SH-SY5Y cells are transiently transfected with the full length (FL) or C-terminal fragment (CTF, a.a. 274–414) of TDP-43 bearing a TC-tag. These cells are then processed for microscopic (biarsenical dye staining, immunostaining and FRET) and biochemical analyses monitoring samples at different time points (24, 48 and 72 hours).

Results: At 24 hrs, FL-TC-FlAsH is distributed uniformly in the cell nucleus. However, after 48 hrs, FlAsH and FRET signals are detected in the cytoplasm, thus suggesting that FL-TC may be mis-localised and accumulates in the cytoplasm when it is overexpressed. Similar to the full-length protein, CTF-TC-FlAsH is initially distributed uniformly in the cytoplasm at 24 hrs, but higher FlAsH signal and FRET signal are detected after 48 hrs. Interestingly, different levels of FRET signal are observed when TDP accumulates, suggesting possible changes in the interactions between the molecules.

Discussion and conclusion: We demonstrate that the TC-tag and biarsenical-labelling technique enables the visualisation of the TDP localization for both the monomers and

aggregated forms within live mammalian cells; in particular, we can monitor the changes in distribution and aggregation as it progresses over time. Such model will prove useful towards investigating how changes in the structure of TDP-43 influence its localisation and aggregation under both physiological and cell stress conditions, and how these processes may relate to the pathogenicity and progression of ALS.

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C94 STAGES OF PTDP-43 PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TDP-43, neuropathology, spreading of pathology

Objective: To see if the distribution patterns of phosphorylated 43-kDa TAR DNA-binding protein (pTDP-43) intraneuronal inclusions in amyotrophic lateral sclerosis (ALS) permit recognition of neuropathological stages.

Methods: pTDP-43 immunohistochemistry was performed on 70 μm sections from ALS autopsy cases (N = 76) classified by clinical phenotype and genetic background.

Results: ALS cases with the lowest burden of pTDP-43 pathology were characterized by lesions in the agranular motor cortex, brainstem motor nuclei of cranial nerves XII-X, VII, V, and spinal cord α-motoneurons (Stage 1). Increasing burdens of pathology showed involvement of the prefrontal neocortex (middle frontal gyrus), brainstem reticular formation, precerebellar nuclei, and the red nucleus (Stage 2). In Stage 3, pTDP-43 pathology involved the prefrontal (gyrus rectus and orbital gyri) and then postcentral neocortex and striatum. Cases with the greatest burden of pTDP-43 lesions showed pTDP-43 inclusions in anteromedial portions of the temporal lobe, including the hippocampus (Stage 4). At all

stages, these lesions were accompanied by pTDP-43 oligodendroglial aggregates. Ten cases with *C9orf72* repeat expansion displayed the same sequential spreading pattern as non-expansion cases, but a greater regional burden of lesions, indicating a more fulminant dissemination of pTDP-43 pathology.

Discussion: pTDP-43 pathology in ALS possibly disseminates in a sequential pattern that permits recognition of four neuropathological stages consistent with the hypothesis that pTDP-43 pathology is propagated along axonal pathways. Moreover, the fact that pTDP-43 pathology develops in the prefrontal cortex as part of an ongoing disease process could account for the development of executive cognitive deficits in ALS.

C95   C9ORF72 REGULATE PROTEIN DEGRADATION PATHWAYS

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Keywords: C9orf 72, endosomal markers, autophagy, heterogeneous nuclear ribonucleic acids

Background: Intronic expansion of a hexanucleotide GGGGCC repeat in the chromosome 9 open reading frame 72 (*C9ORF72*) gene was identified as the major cause of familial amyotrophic lateral sclerosis (ALS).

Objective: Elucidation of C9ORF72 normal cellular function in cellular trafficking and protein degradation.

Methods: We investigated the role of C9ORF72 in protein trafficking in neuronal cell lines, using reporter constructs, immunofluorescence microscopy, immunoprecipitation and mass spectrometry to identify the most abundant proteins that interact with C9ORF72. We also performed immunohistochemistry on C9ORF72 positive ALS patient tissues.

Results: We demonstrated that C9ORF72 was expressed in both the nucleus and cytoplasm as vesicular-like structures, and it was also secreted into the extracellular space. C9ORF72 co-localized strongly with Rab 1, 5, 7 and 11, consistent with previous studies that it bears Rab guanine exchange factor (GEF) activity. A physical interaction between C9orf72 and these Rab proteins was confirmed using co-immunoprecipitation studies. Depletion of C9ORF72 using siRNA inhibited transport of Shiga toxin from the plasma membrane to Golgi apparatus, revealing that C9ORF72 regulates endocytosis. Immunohistochemistry of C9ORF72 ALS patient motor neurons revealed increased co-localisation between C9ORF72 and Rab 7 and 11 compared to control patients, indicating dysregulation of Rab-mediated trafficking in patients bearing the C9ORF72 intronic mutation. C9ORF72 also co-localised with markers of the autophagosome and siRNA treatment altered the ratio of LC3I:LC3II, demonstrating that C9ORF72 regulates autophagy-related endosomal trafficking. Investigation of proteins interacting with C9ORF72 using mass spectrometry identified two other proteins linked to ALS, ubiquilin-2 and heterogeneous nuclear ribonucleoproteins, hnRNP A2/B1 and hnRNP A1. Treatment of cells over-expressing C9ORF72 with proteasome inhibitors induced the formation of intra-nuclear aggregates of C9ORF72 suggesting that proteasome activity alters C9ORF72 cellular distribution.

Conclusion: This study demonstrates that C9ORF72 regulates endolysosomal trafficking and protein degradation pathways in neuronal cell lines. Furthermore, these data demonstrated that C9ORF72 is associated with heterogeneous nuclear ribonucleic acids (hnRNPs), suggesting that C9ORF72 may have a role in RNA metabolism and transport.

SESSION 10B RESPIRATORY AND NUTRITIONAL MANAGEMENT

C96 ASSESSMENT AND MANAGEMENT OF CALORIC NEEDS IN ALS

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Keywords: malnutrition, caloric balance, enteral feeding

Caloric under nutrition or marasmus is common in ALS and although multifactorial, largely results from caloric imbalance due to decreased intake or increased expenditure, singly or in combination. The adverse effects of malnutrition include loss of weight and BMI, alterations in body composition, decreased survival, and possible effects on Quality of Life (QOL) and general motor and respiratory functions (1). Decreased caloric intake has been reported in up to 90% of patients during the disease course (2). Increased caloric expenditure is suggested by 12–20% and 27% higher measured than calculated resting metabolic rate in about 48–62% of sporadic and 100% of familial ALS, respectively, suggesting intrinsic hypermetabolism (3). The hypermetabolism correlated with age, gender and fat-free mass. Nevertheless, other causes of increased caloric use have not been explored, particularly physical activity caloric expenditure and non-exercise activity thermogenesis (NEATS) that are germane to ALS, and could result from weakness, spasticity, fasciculations, cramps, emotional lability and respiratory insufficiency. In the late stages of ALS, however, patients become hypometabolic especially after gastrostomy and mechanical ventilation support (4).

Routine enquiry about nutritional habits and caloric intake at regular visits, and recording of weight, ALSFRS-R scores, FVC, and employment of a 'nutritional' equation such as the Harris–Benedict equation, are advocated (5). Recommended surrogate indicators of the need for caloric supplementation include weight loss of 5–10% over healthy state, symptomatic dysphagia and FVC less than 50% of predicted; however, none of these criterion takes into account caloric balance, particularly caloric needs. Equations currently used to predict caloric needs are imprecise and inaccurate as they do not account for the variable topography and severity of the disease, and resultant variations in physical activity (6). We have developed a modified equation which can be used in clinics, validated against caloric needs measured directly by the gold standard doubly labeled water technique, to accurately predict caloric requirements in patients with different severity and phenotype of the disease (7).

Malnourished patients are encouraged to maximize oral nutritional rehabilitation, and when unsuccessful caloric supplementation is recommended through an enteral tube, such as a percutaneous endoscopic gastrostomy (PEG) or radiologically inserted gastrostomy (RIG) (8). Morbidity and mortality are slightly lower for RIG than PEG tubes, although further study is needed. Successful enteral feeding stems weight loss, and improves QOL and possibly survival in patients (9). Formal criteria for timing, rate and extent of caloric replacement are needed. Further, caution needs to be exercised to prevent the development of the refeeding syndrome, with its antecedent effects of enhanced morbidity and mortality (10).

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C97 A MULTI-CENTRE EVALUATION OF SECRETION MANAGEMENT IN PATIENTS WITH MOTOR NEURONE DISEASE (MND)

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Keywords: saliva, management, symptoms

Background: Excessive saliva is an unpleasant and challenging to treat symptom in MND. Consequential problems include drooling, embarrassment, higher risk of aspiration and the soiling of clothes. There is little evidence to guide clinicians when managing these problems and a recent Cochrane review highlighted the need for more studies.

Objectives: To carry out an evaluation of the management strategies that are being used to treat excessive saliva by clinicians treating MND at the Sheffield MND clinic.

Methods: Retrospective case note review of deceased patients who attended the MND clinic in Sheffield between 2002 and 2012.

Results: The case notes of 518 individuals with MND were reviewed. Problems with excessive saliva were identified in 266 patients (51%). Management for excessive saliva was described in 186 cases; seven anticholinergics were administered in 11 preparations and 91 doses. Hyoscine and amitriptyline were the most common therapies, used in 164 and 91 patients, respectively. Botulinum toxin was used in 45 patients.

Hyoscine patches were the anticholinergic that was most often effective. The response to hyoscine was documented in 79 patients; symptomatic relief was experienced in 67 (85%). Of the patients who were benefitting from hyoscine, 34 (51%) required additional management as their disease progressed, 13 (19%) of whom had to discontinue the hyoscine patches because of adverse effects. In 12 patients (18%) the adverse effect responsible for the discontinuation was a skin reaction.

Neurobloc botulinum toxin was given to 35 patients. The outcome of injecting both the submandibular and parotid glands was recorded in 21 patients. Eighteen (86%) had some symptomatic improvement. The outcome of injecting only the parotid glands was recorded in six patients, two patients (33%) symptoms improved. The comparative incidence of adverse effects in those who did and did not have their submandibular glands injected were nines patients (47%) and one patient (13%), respectively.

Discussion and conclusion: Hyoscine patches are the most effective anticholinergic for reducing excessive saliva and finding ways to manage the skin reactions to these patches may allow more patients to better control their symptoms. The benefits of hyoscine patches seem more limited as a patient's symptoms progress, and more invasive therapies such as salivary gland botulinum toxin injection may be required.

Neurobloc injections solely into the parotid glands are less often successful than injections involving the submandibular glands. Patients with troublesome excessive saliva may require injections into their submandibular glands to experience a benefit, whilst patients with milder symptoms could benefit from injections solely into the parotids.

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C98   ORAL SECRETION SCORE (OSS) PREDICTS BEST CARE INTERVENTIONS AND OUTCOMES OF PATIENTS WITH ALS/MND USING NONINVASIVE VENTILATION (NIV)

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Keywords: noninvasive ventilation, oral secretions, airway management

Background: Adherence to NIV in ALS/MND patients is limited by worsening oral secretions. The Oral Secretion Score (OSS) developed to assess oral secretions has been validated in ALS patients from USA, Japan, and France (1,2,3).

Objective: To relate OSS to compliance with treatment interventions in ALS patients requiring NIV.

Methods: This prospective observational study involved 135 consecutive ALS/MND patients (M: 51%: F: 49%; median age 62 years; range: 31–86 years) in Ohio, USA who were followed prospectively during home visits from start of NIV until they died or transitioned to tracheostomy.OSS, categorizing the severity of oral secretions in relationship to changes in swallowing and coughing (4 = normal automatic saliva swallow; no drooling; 3 = minimal drooling; automatic saliva swallow decreased; 2 = moderate drooling; conscious swallow required; 1 = severe drooling; difficult conscious swallow; ineffective cough force; 0 = most severe drooling; conscious swallow impossible;no cough force), was measured at beginning of NIV and throughout follow-up in clinic and home visits. Adherence to NIV, tolerance to NIV, and effective use of Mechanical In-Exsufflation (MIE) related to OSS were assessed longitudinally. Time to failure analysis of compliance with NIV as a function of OSS was evaluated by standard statistical evaluations (MedCalc Statistical Software www.medcalc.org).

Results: At start of NIV, 57% (77/135) patients had no bulbar impairment and (OSS = 4). Of the rest, 43% (58/135) had bulbar signs (13%(17) (OSS = 3), 18%(24) (OSS = 2), and 13%(17) (OSS(= 1)). Median time on NIV for the 135 was significantly longer in ALS patients (OSS = 4) (10.3 months; 95% CI = 8.0–14.0 months) compared with (OSS < 4) (3.1 months; 95% CI = 2.1–4.0 months); $p < 0.001$). NIV was better tolerated in 118 patients (OSS = 2–4) significantly compared with 17 patients (OSS = 1) (Chi Square = 135; $p < 0.01$). MIE was used for airway clearance by 16% (22/135). Of these 50% (11/22) (OSS = 0–1) were intolerant and discontinued its use, while 50% (11/22)-7 (OSS = 4)-4 (OSS = 2–3) used MIE effectively to remove phlegm/thick mucus Chi Square = 22; $p < 0.01$). Pharmacological agents were used to reduce sialorrhea in 44% (60/135). In 40% (54/135) who had (OSS = 2–3), patients reported drugs were effective in reducing saliva, but not if (OSS = 0–1). Oropharyngeal suctioning was used to clear upper airway in 38% (51/135). In 29% (39/135) who had (OSS = 2–3), patients indicated suctioning was effective in clearing airway, but ineffective in maintaining airway if (OSS = 0–1).

Conclusions: Higher OSS at initiation of NIV is significantly associated with improved tolerance/adherence to NIV and continued use of this intervention. Noninvasive airway clearance is not achieved in patients (OSS = 1). Saliva control (via medications) and expectoration control (via MIE) was significantly more effective when (OSS = 2–4). Replication of these findings in additional patient cohorts may establish the usefulness of the OSS in disease management of patients with ALS undergoing NIV intervention.

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C99 NOCTURNAL TRANSCUTANEOUS CAPNOGRAPHY IN ALS IS A RELIABLE AND NON-INVASIVE PARAMETER FOR DECIDING NON-INVASIVE VENTILATION IN ALS PATIENTS

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Background: Non-invasive ventilation (NIV) may improve quality of life as well as survival in ALS patients. There is an agreement about the importance of using NIV as early as possible. However, the best criteria for starting NIV remain in dispute.

Objectives: To study the usefulness of nocturnal transcutaneous capnography (NTC) as a parameter for deciding NIV in ALS.

Methods: We studied clinical characteristics (age and site of onset, ALS duration, and ALSFRS score) and respiratory data (SVC, FVC, PImax, SNIP, nocturnal oxymetry, PaO_2, and $PaCO_2$) of patients with definite ALS, that had NTC between March 2012 and March 2013, as well as their outcome after the NTC exam (delay for starting NIV).

Results: There were 21 patients, 8 women and 13 men, with a mean age of 62.6 yrs. Mean disease duration was 22.7 months, six patients had bulbar onset, 15 had a spinal onset and mean ALSFRS score was 35.4/48. At NTC, the median PCO_2 of the population was 48mm Hg. We compared the groups below and above this median, comprising 10 and 11 ALS patients, respectively. At the time of submission, 100% of the patients with high PCO_2 are now under NIV *vs* 10% in the other group. Mean delay for NIV in the first group was 6.55 months. Comparison between two groups for clinical characteristics and respiratory data allowed only one significant difference to be noted: SNIP was significantly lower in patients with hypercapnia (23% vs 52, p = 0.01).

Discussion: Hypercapnia is one criterion for NIV in ALS patients. This measure is an invasive one, while NTC is non-invasive and may be obtained at home (this is the case for all the patients described here). Our data suggest that this measure is an option for following respiratory involvement in ALS patients and that it may help for the decision of NIV. It appears to be as pertinent as the SNIP but SNIP measure may be difficult to obtain/interpret particularly in bulbar patients.

Conclusion: The measure of PCO_2 transcutaneously is a reliable, easy to obtain and non-invasive technique. We believe that it has to be considered as an additional criterion for NIV decision.

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C100 CAN NIV PARAMETERS SETTINGS AND CHANGES OVERTIME PREDICT FUNCTIONAL AND SURVIVAL OUTCOME IN ALS PATIENTS?

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Background: Early NIV may play a critical role in survival, and in its compliance. However, adherence have only been evaluated regarding the number of hours of use, and yet others variables recorded in the equipment's software could also be useful.

Objective: We looked for potential predictors among ALS patients fully compliant to NIV.

Methods: In a prospective trial design, we followed-up 60 ALS patients from January 2008 to May 2012 that at the end of study were ascribed to two groups according to whether they were dead (G1) or alive (G2). They were all early ventilated, based on abnormal oximetry, phrenic nerve response or ALSFRS-R score less than 12. At each 3 months, patients were extensively evaluated with respiratory function testing and all data from the NIV equipment were downloaded and registered. Primary outcomes: ALSFRS functional decline and disease duration to death or end of study; Secondary outcomes: time to NIV; NIV use and parameters settings at NIV adaptation.

Results: No clinical or demographic differences were observed between groups at admission. Disease duration from symptoms onset and time to NIV adaptation were also non-significant, but disease duration correlated positively with maximal inspiratory pressure, IPAP and backup breathing rate and nocturnal SpO_2. At the end of study, there were significant differences showing better functional status in G2 with lower heart and breath rate and reduced time spent with lower SpO_2 and have higher IPAP pressures. Multivariate Cox regression analysis showed that IPAP pressures more than 18 cm H_2O was a significant preditor of survival (-2 Log-L 82.56; sig. 0.0004; B$-0,36$; CI-0.53 to 0.77; sig. 0.02) and a trend regarding a lower respiratory decline (-2 Log-L 124.03; sig. 0.000; B-2.46; CI 0.53–0.77; sig. 0.05).

Conclusions: For the first time, determinants of functional decline and survival are significantly related to parameters settings of NIV equipment.

C101   DIAPHRAGM FUNCTIONAL ANALYSIS AT THE UPPER AND LOWER SPECTRUM OF FORCED VITAL CAPACITY (FVC) IN ALS/MND: FVC INADEQUATELY ASSESSES DIAPHRAGM FUNCTION OR UPPER MOTOR NEURON INVOLVEMENT FOR STIMULATABILITY

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Keywords: FVC, diaphragm, diaphragm pacing

Background: Forced vital capacity (FVC) is widely used as an indicator of prognosis in ALS, need for non-invasive ventilation (NIV), diaphragm pacing (DP) and inclusion/assessment of efficacy in clinical trials. Clinical trials may only accept patients above a FVC of 65% predicted yet these patients may have significant diaphragm dysfunction that could affect study results. Also patients with FVC greater than 65% may need diaphragm dysfunction addressed with NIV or DP. Patients with a FVC below 45% may be excluded from DP or gastrostomy tubes yet have a stimulatable diaphragm.

Objective: Analyze patients with FVC's greater than 65% or less than 45% to determine the relationship between FVC and other diaphragm functional tests including assessing operative diaphragm response with direct stimulation.

Methods: Retrospective analysis of a prospective database at a single site under an IRB protocol after FDA approval in November 2011.

Results: Ninty-six ALS subjects were evaluated with 86 patients undergoing DP (10 had non-stimulatable diaphragm at surgery or with pre-operative testing with FVC ranging from 28% to 65%). Twenty-nine patients had a FVC above 65% (average: 79, range: 65–110). This subgroup 26 (90%) had at least one additional test: 23 (79%) had a MIP less than 60; 18 (62%) had an elevated hemidiaphragm on CXR; 23 (79%) had reduced diaphragm excursion of 2 cm or less on fluoroscopy; 15 (52%) had a measured amplitudes of less than 0.2 on phrenic nerve conduction study; five (17%) had hypercarbia with pCO_2 above 45; and three patients had sniff with paradoxical diaphragm motion (FVC of 92%, 79% and 73%). Twenty-four patients had FVC 45% or less. All had MIP less than 60 (average: 23, range: 11–46). All had a stimulatable diaphragm at surgery with 80% having simultaneous feeding tube. There was no peri-operative mortality. Using regression analysis sniff fluoroscopy of diaphragm function had the greatest correlation to MIP (r = 0.47) then CO_2 levels (r = 0.33) then FVC with the weakest (r = 0.29). In operative assessment of diaphragm stimulatability; in those with FVC greater than 65%, 70% measured strong diaphragm response and in those below 45% FVC group, 50% had the strongest diaphragm rating. Intra-operatively, three patients with the weakest diaphragm score had FVC greater than 80%. Survival analysis based on each one of these functional tests is ongoing.

Discussion and conclusion: In ALS, a FVC above 65% is considered adequate yet many can have considerable diaphragm dysfunction. If this is identified and the diaphragm has primary upper motor neuron involvement, then therapy such as DP can be used to improve diaphragm function, respiration and in unilateral cases prevent paradoxical movement which leads to atrophy, muscle elongation and damage. Patients with ALS and FVC below 45% can still have significant intact lower motor neurons with stimulatable diaphragm muscle units where DP can be utilized. ALS patients need a thorough analysis of diaphragm function to assist in prognosis and management of their disease.

C102   AN AMBULATORY MODEL OF NON-INVASIVE VENTILATION IMPLEMENTATION IMPROVES SURVIVAL IN MOTOR NEURONE DISEASE

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Keywords: non invasive ventilation, ambulatory model of care, survival

Background and Objective: Non-invasive ventilation (NIV) increases survival and quality of life in Motor Neurone Disease (MND) (1). Historically at our institution, NIV was implemented during a multi-day inpatient admission, but increased demand lead to prolonged waiting times. We developed and implemented an ambulatory model and report the prospective evaluation of its impact.

Methods: All people with MND referred for implementation of NIV 6 months before and after the ambulatory model commenced were included. The ambulatory model involved a 4-hour stay to commence ventilation, including mask fitting, bedside titration of the spontaneous-timed mode bi-level pressure ventilator (VPAP III ST and ST-A, Resmed, San Diego USA) and education. Follow-up consisted of in-laboratory polysomnography and outpatient attendance. Waiting time, hospital length of stay, adverse events and polysomnography data were reviewed before and after the model change.

Results: Twenty-nine patients were included in the analysis, with similar baseline characteristics (mean (SD) FVC% pred 51.8 (15.2) pre- vs 54.2 (17.4) post-model change, p = 0.72). After changing to the ambulatory model the median waiting time to commence ventilation fell from 33 to 14 days (p < 0.04) and adverse events reduced (4 of 17 (3 deaths, 1 acute admission) pre vs 0 of 12 post). Survival was also prolonged (median (IQR) 278 (51–512) vs 580 (306–1355) days; hazard ratio 0.41, p = 0.04). Despite poorer sleep quality on follow-up polysomnography, daytime CO_2 was not different.

Discussion and conclusion: The introduction of an ambulatory model to commence NIV resulted in more rapid initiation of ventilation with an associated improved survival in our tertiary domiciliary ventilation service. This model of care provided an efficient option for implementing NIV, with waiting time reducing by 19 days and a 24% reduction in adverse events. Efficacy of ventilation, as measured by daytime $PaCO_2$ was similar. The flexibility of the ambulatory care model provided for prompt scheduling of NIV trials in patients who were deteriorating regardless of inpatient bed capacity.

Criteria for referring for ventilation did not change during the audit period; patients post-model change did not appear to have been referred earlier in their disease course; however, they were commenced on ventilation more quickly once the decision to ventilate was made. Our data suggest that once a decision to ventilate has been made, delays in commencing NIV are clinically important and that alternative models of implementation can be effective.

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SESSION 11 JOINT CLOSING SESSION

C103   THE FUTURE OF ALS THERAPEUTICS

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Despite multiple trials and many promising leads, the development of new therapeutics for ALS has remained challenging. Yet, the future of drug development for ALS is encouraging. The vast amount of genetic data has defined and will continue to define new targets for therapeutics. Novel tools to modulate these targets will turn an increasing number of these discoveries into new drugs. Methods for targeted therapies include small molecules, passive immunization, siRNA, and antisense oligonucleotides. Each of these will be discussed, with a focus on recent experience with antisense oligonucleotides. Antisense oligonucleotides designed to lower SOD1 mRNA and protein were recently shown to be safe in a Phase I trial for SOD1-related ALS. The pharmacokinetic data in humans from this study will be helpful for conducting future antisense oligo trials. Antisense oligonucleotides strategies may be used to lower other mRNAs, change splicing isoforms, or to inhibit regulatory RNAs such as miRNAs. Inhibition of the regulatory miRNA, miR-155, for example, extends survival in the ALS model SOD1^{G93A} mice. Targeted therapies for distinct populations of patients with ALS have now become possible and are likely to become increasingly important. Some of these therapies developed for a small subset of ALS, may be worthy of testing in a broader group of ALS patients especially where shared pathophysiology may be demonstrated.

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THEME 1 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

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THEME 1 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P1   MULTIDISCIPLINARY ALLIED HEALTH PRACTICE GUIDELINES FOR PHYSICAL, SPEECH AND OCCUPATIONAL THERAPY IN ALS

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Keywords: multidisciplinary, guidelines, quality of life

Background: Evidence-based clinical practice guidelines improve quality, effectiveness and appropriateness of patient care. For ALS, such guidelines are not available for the allied health care professions.

Objectives: The aim of this project was to systematically develop guidelines for the multidisciplinary rehabilitation management of patients with ALS. The recommendations will support physical, speech and occupational therapists in clinical decision making with respect to the diagnosis, treatment and evaluation of relevant impairments and restrictions in functioning, activities and participation of patients with ALS.

Methods: A taskforce was formed and they started the project. The International Classification of Functioning, Disability and Health (1) formed the base. The guidelines were developed according to the model from the Dutch Institute for Healthcare Improvement (CBO) 'Evidence based guideline development' (2). This model contains the following steps: systematic literature search, quality assessment and summary of the evidence; formulation of concept recommendations; feedback from experts; rephrase of recommendations; final approval and authorisation by relevant stakeholders.

Results: The project resulted in practice guidelines with recommendations regarding diagnostics, intervention and evaluation of functioning within the relevant domains of the International Classification of Functioning, Disability and Health (1). The recommendations were developed for physical, speech and occupational therapists concerning all rehabilitation stages integrating evidence from research and clinical expertise of expert health care professionals, patients and their carer's preferences, national associations of physical therapy, speech therapy and occupational therapy and of the Netherlands ALS Center. In addition to the practice guidelines and the recommendations, a summary of the clinical reasoning process in algorithms is provided. The guidelines are e-published in Dutch and available at www. als-centrum.nl/als-richtlijn/ with free downloads.

Discussion and conclusion: The development of the multidisciplinary guidelines has allowed recommendations on diagnostic and therapeutic interventions for physical, speech and occupational therapists working with patients with ALS. Implementation of the guidelines will improve standardization and transparency of the diagnostic and therapeutic process. As the guidelines were developed simultaneously for these three allied health care professions, interdisciplinary aspects of treatment are covered as well which will improve care and quality of life for patients with ALS. Implementation of these guidelines is the next crucial step to take.

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P2   DEVELOPING A REMOTE MULTIDISCIPLINARY CLINIC: INITIAL OBSERVATIONS AND LESSONS LEARNED

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Keywords: remote clinics

Background: Bringing expert care to ALS patients residing in rural areas has been a frequent topic of discussion. A sustainable plan has not been enacted and tested for its cost and effectiveness. The situation is especially challenging in the Appalachian region of the US where poverty and geographic distance conspire to restrict access to tertiary care. Various approaches have been tried including personal travel by the ALS neurologist to a remote site or connecting via telemedicine links to directly evaluate the patient. Both approaches are expensive in terms of professional time and money, and therefore, lack sustainability. More importantly, the expertise of a team of regional therapists (PT, OT, RT, etc.) cannot be cultivated easily with these models.

Objectives: To establish a remote multidisciplinary clinic in eastern Kentucky (termed a Virtual ALS Center or VALSC), develop the expertise of regional therapists, communicate via telemedicine links, and measure the clinical effectiveness of this approach.

Methods: Our ALS Center at the University of Kentucky (UK) established a partnership with Our Lady of Bellefonte Hospital in Ashland, KY (approximately 125 miles, or 201 km distant) which agreed to host the multidisciplinary clinic staffed by their therapists. Regional ALS patients will be evaluated and treated in the VALSC in accordance with the AAN Practice Parameters.

We will identify a group of pre-intervention ALS patients who were residents of the target Appalachian region during 2005–12 and evaluate medical records to determine adherence to the AAN Practice Parameter. Their care will be compared to matched ALS patients attending our UK ALS center. The ongoing effectiveness of the VALSC will be determined by its adherence to the AAN Practice Parameter, the time and distance saved by patients attending the VALSC, and cash flow. Comparisons of the VALSC with other ALSA Certified Centers will be conducted.

Results: Development of infrastructure and training of the therapists for the VALSC has been accomplished. Approximately 40–60 historical ALS patients from the target region have been identified. The VALSC will begin operation in July 2013 and initial results will be presented.

Discussion and conclusion: The success of an enduring VALSC will depend on finding an institutional partner with shared values with the ALS Centers in the care of complex patients with nonsurgical illness. The partner needs to provide dedicated space and sufficient time commitment to develop the expertise of the local therapy team and neurologist. Telemedicine links to the tertiary ALS Center is critical for consultation and training. Issues of licensure, credentialing, privileging, and medical insurance are critical. Finally, the VALSC needs to be at least revenue-neutral for the host partner.

P3　MULTIDISCIPLINARY TEAMS: EXCELLENT CARE FOR PATIENTS, BUT HOW DO WE CARE FOR OURSELVES?

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Keywords: bereavement, coping, grief response

Background: Caring for patients with life-threatening diseases can elicit grief symptoms in health care professionals. Limited descriptions exist of how individually and collectively, MND/ALS teams can safely express grief and model effective coping strategies.

Objectives: To explore mechanisms for managing patient loss and describe beneficial components of supportive models of intervention.

Methods: Individual interviews were conducted with healthcare team members to: identify challenges of work-related bereavement; current management of grief and loss; and ways of supporting interpersonal functioning and resiliency. Data collection involved a nine open-ended question instrument to elicit responses regarding emotions, experiences, perceptions, and grief resolution strategies.

Results: Over 28 months at The Methodist Neurological Institute, 176 new ALS patients were diagnosed and 124 deaths were reported. MDA/ALS Team Hope is comprised of 25 health carers in addition to staff caring for patients when hospitalized. Twenty-two individual interviews were completed. Responses were categorized into group and individual strategies. Group strategies included recognizing patient death by sending email to team; sending team signed card; attendance at funerals; discussing and reminiscing among team members; and staying in touch with family through ALS fundraisers. Individual ways of managing grief included sending private cards or emails; private tears; prayer; exercise; and journaling. Members indicate level of grief response often corresponded to: depth of their relationship with patient; similarity of patient to themselves in age or gender; and unexpected versus expected death. Grief response increased with frustration of late diagnosis, noncompliance with recommendations, and/or inappropriate treatment recommendations before patient arrived at the Institute. Members reported unique aspects of ALS-diagnosed population (positive outlook, giving spirit, and inspirational) as increasing grief response while simultaneously motivating professions to "do more" and "find a cure."

Discussion: This descriptive exploration indicates the health care team is informally and formally providing a safe environment for grief responses following patient death. Having a team member conduct the interviews seemed to elicit deeper, more personal, and possibly cathartic responses. Majority indicated satisfaction with strategies as providing closure and validation of role and grief. Four individuals suggested adding "lessons learned" component to strategies. Grieving was recognized as a bonding experience; enhancing their ability to re-invest energy in patients. Dysfunction or burnout does not appear prevalent.

Conclusion: Further studies are needed and should include description of numerous MND/ALS health care teams to provide an outline of essential grief and coping components. With a grief and coping algorithm, teams can enhance their system for providing expression of normal grief responses. As health care professionals, developing some level of comfort with the issues of life and death and learning to live with our grief increases our ability to administer life-enhancing care, even to patients with a life-threatening disease. Learning self-care is an essential component in this process.

P4　PATIENTS' AND PROFESSIONALS' PERSPECTIVES ON CASE MANAGEMENT IN ALS CARE

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Keywords: case management, care, patients'/caregivers'/ professionals' perspective

Background: The concept of case management has been suggested as an innovative strategy to optimize complex multidisciplinary care in patients with ALS and their caregivers. In the absence of high quality evidence on the effectiveness of case management in ALS care, a cluster-randomized controlled trial (RCT) on case management in Dutch

patients with ALS and their caregivers was conducted and showed no effect of case management on quality of life of ALS patients or on caregiver strain. More insight in the experiences of patients with ALS, their caregivers and health care professionals with case management may explain these negative results.

Objective: To explore the experiences of patients with ALS, their informal caregivers and health care professionals with case management in ALS care.

Methods: A qualitative study, nested in a large cluster-RCT on the effectiveness of case management. In-depth, narrative interviews with 10 ALS patients and their primary informal caregivers were held after 12 months case management. Also, 10 interviews were held with ALS health care professionals. Beside the interviews, a heterogeneous focus group was held with patients, informal caregivers and health care professionals. The data were audio taped, fully transcribed and analysed thematically by two independent researchers.

Results: Patients, informal caregivers and health care professionals noted that in certain circumstances case management may be of added value. Important factors were the adequacy of usual care. Also, a faster rate of progression of the disease was noted as an important factor, both by patients and professionals. Therefore, patients and health care professionals concluded that the need for case management additional to usual multidisciplinary care differs between patients.

The most important elements of the case management that were appreciated by patients and by health care professionals were the fact that the case manager did not work on a tight schedule and did not visit the patients and their caregivers at home.

Besides these elements, patients and caregivers pointed out that they valued the mental and practical support from the case manager, that is, to reflect on the situation with the case manager or to get help with applications for devices. However, the health care professionals pointed out that the need for additional practical support depends on the adequacy of usual care provided by the multidisciplinary team.

Conclusion: The study shows that a qualitative exploration of experiences with case management from different perspectives has made it possible to arrive at a greater understanding of the care needs of patients with ALS and their caregivers. Patients and health care professionals agree that in ALS care custom-made support is the key to care that best meets the needs of patients with ALS and their caregivers.

P5   COMPREHENSIVE CARE AND HOME TELEHEALTH FOR VETERANS WITH ALS

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Background: The Veterans Administration (VA) has been on the forefront of telemedicine for delivering care to veterans in rural communities. Veterans with ALS (VALS) often

have limited access to specialty clinics because of impaired mobility, transportation of medical devices, and long travel times. Comprehensive multidisciplinary care for ALS has been effective for improving quality of life (QOL) and in some cases extending survival. VRHRC-ER in partnership with the North Florida/South Georgia Veterans Health System (NF/SG VHS) established a collaborative approach to comprehensive ALS care that includes home Clinical Video Telehealth (CVT) and provides close observation of VALS for decline in function and increased disability.

Objective: To demonstrate that home telehealth can be successfully implemented into the multidisciplinary management of VALS. In addition, we hope to determine if this data can be replicated in a larger study with additional patient and caregiver specific outcomes.

Methods: Home CVT was used to augment multidisciplinary care for VALS in NF/SGVHS. The comprehensive ALS care team included a neurologist, mid-level medical provider, physical therapist, occupational therapist, speech therapist, and social worker. After initial, and in some cases, follow-up face-to-face visits, VALS were given the option of using a home CVT unit immediately or when decline limited their ability to travel for follow-up visits scheduled at 3-month intervals. Outcome measures included weight loss, functional decline, detection of respiratory decline, reduction in hospitalizations, and reduction in caregiver burden.

Results: In the first 6 months, 17 VALS were enrolled at the NF/SGVHS ALS clinic. Eight were classified as rural or highly rural living greater than 2.5 hours from the ALS clinic. All 17 had an initial pulmonary function screening and PT assessment. To date, 109 Home CVT visits were conducted, saving 28,498 miles and nearly 525 hours of travel time by VALS and caregivers. Reimbursable mileage costs were reduced by $12,111. Data on functional decline, hospitalization, and caregiver burden will be presented.

Discussion and conclusion: Home telehealth in the care of VALS demonstrated improved patient satisfaction, reduced driving time, and reduced total cost and can be a valuable tool for patients who have limited access to specialty care. This demonstration project will be expanded to comprehensive ALS care clinics in New England (Providence, RI; West Haven, CT; and White River Junction, VT). The additive effect of home CVT on improving QOL, increasing survival, and decreasing caregiver stress will be determined. If successful, this model of ALS care may be available to all VALS in the USA.

P6   THE COST OF MANAGING ALS IN A TERTIARY REFERRAL CLINIC: A RETROSPECTIVE CHART REVIEW

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Background: People with ALS are often intense users of medical services; however, relatively little is known about the cost of providing such services. Quantifying the cost of ALS services is important as it gives an indication of the potential

savings arising from the development of treatments which slow down the progression of the condition.

Objectives: The aim of the study was to estimate the cost of managing ALS in a tertiary referral clinic in Ireland.

Methods: The analysis was based on a retrospective analysis of the medical charts of 119 representative patients who attended a tertiary referral clinic and who died between 2010 and 2013. Information on number of clinic visits, number of visits to allied health professionals, hospital admissions, procedures and aids, and home visits were recorded. Each resource was assigned a unit cost to provide the overall cost of providing care within the clinic. Costs were analysed separately by site of onset.

Results: 54% were male and mean age of onset was 64 years. 34% had bulbar onset disease. The mean length of time between the onset of symptoms and the first clinic visit was 13 months; while the mean survival time from first clinic visit was 21 months. The mean number of patient visits was 6 (minimum: 1 visit and maximum: 36 visits). 9% did not return to the clinic after the first visit, while 17% visited the clinic 10 or more times. The majority of patients were seen by allied health professionals during their clinic visit. For example, at their first clinic visit, 27% were referred to speech and language therapist, while 45% were referred to an occupational therapist. In addition, 51 patients received at least one home visit from the ALS clinical nurse specialist; while one patient received 10 such visits. Patients were in receipt of a wide range of interventions and aids including non-invasive ventilation and communicative and walking aids. A unit cost will be assigned to each of the resources which are used to provide an indication of the average cost of managing a patient within the clinic.

Discussion: Accurate estimates of the cost of care will permit modelling of the economic implications of symptomatic and disease modifying interventions.

P7 THE ROLE ANALYSIS OF THE COORDINATORS FOR PATIENTS WITH INTRACTABLE DISEASES IN JAPAN FROM THE POINT OF VIEW OF CONTINUITY OF CARE

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Keywords: coordinator, intractable neurological disease, continuity of care

Backgrounds: In the 1970s, the Ministry of Welfare, Japan, adopted a law to support those who had intractable diseases

such as ALS. In 1998, prefectural medical health care networks were initiated to improve the care for patients with seriously intractable neurological diseases, especially those with ALS on artificial ventilation. Fifty-five medical care coordinators are currently introduced in 33 prefectural networks to support those patients. These coordinators need to have not only medical knowledge, but also information about the local welfare and healthcare system, and skills to cooperate closely with multidisciplinary teams. As they are employed on variable terms and conditions, most coordinators have few opportunities to have training about the expertise. Our previous study showed that most coordinators strongly felt the importance of training.

Objective: We attempted to define the roles of and the competencies for the coordinator and to make models for care coordination through case examination.

Methods: We collected the experiences for successful management from coordinators by e-mail. Successful management means that coordinators could adjust the care service taking patients' QOL, and patients' and their families' needs into consideration. We classified these experiences into the following subjects: (1) management of the transfer to chronic care hospitals; (2) management of admission for respite care; (3) coordination of home care services; and (4) coordination of medical care system when patients move their residence to another prefecture. We used the Freemann's model of 'continuity of care' as the analytic frame for our study. The model was developed through people with long-term neurological conditions in the UK. The definitions comprise a number of elements such as (1) longitudinal continuity, (2) relational, personal and therapeutic continuity; (3) long-term neurological conditions; (4) cross-boundary continuity; (5) flexible continuity; (6) information continuity; (7) social context; and (8) personal agency. This concept is supposed to clarify the coordinator's roles and provided a way of examining the effects of integrated services.

Results: Twenty-one cases were collected from 18 coordinators. The response rate was 32%. According to qualitative case analyses, in almost all cases coordinators implemented the elements such as 'longitudinal continuity'; 'relational, personal, and therapeutic continuity'; 'cross-boundary continuity'; and 'information continuity'. In 50% of cases coordinators achieved 'personal agency' and 'long-term neurological conditions', in 30% cases 'flexible continuity', and in 25% cases 'social context'.

Conclusions: Coordinators made a significant contribution to integrate services by medical and health professionals for patients with intractable diseases. The coordinate models are required to accomplish continuity of care for patients. Through case examination we can specifically share and improve approaching strategy to facilitate the continuity of care for patients. Using the concept of continuity of care, coordinators could recognize the expected role, evaluate their activity, and improve their faculty.

P8   THE TRACE TO THE FIGHT FOR THE IMPROVEMENT IN PALLIATIVE CARE OF THE PATIENTS WITH ALS IN JAPAN

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Keywords: palliative care, morphine, survey of ALS physicians

Background: Despite the fact that morphine prescription is a standard therapy for the end-of-life care of ALS in Western countries, it is not popular in Asian countries and other areas. Unlike cancer patients, the long-acting morphine and other opioids, and hospice care for ALS used to be outside of the insurance coverage in Japan. All we could do was to try to persuade the authority to make an exception or medical providers like us paid for the patients. In 2009, we conducted the survey on physicians treating ALS in Japan, and reported that only 21% of the respondents would prescribe morphine to their ALS patients under these circumstances.

Objective: The purpose of this paper is to share our experiences with other countries having difficulty in prescribing morphine for ALS patients.

Methods: For the past 3 years, we tried several projects to improve this situation to protect patients' right. First we published a handbook on the palliative care for ALS, second we gave lectures on the palliative care for ALS many times throughout Japan, third we worked toward the Ministry of Health, Labor and Welfare to change the policy to make morphine for ALS covered by insurance.

Finally, in September 2011, hydrochloric acid morphine and morphine sulfate for ALS were covered by the national insurance. Therefore, we conducted the second survey on the physicians treating ALS to investigate any change occurred since 3 years ago. We sent out questionnaires to all of board-certified neurologists, same as the first survey.

Results: One thousand and fifty-three responded (response rate 22%) and their background was the same as the first survey. The percentage of answered respondents who prescribed morphine to their ALS patients was 32% (21% in first survey). The respondents who have prescribed morphine to more than five patients increased from 23% to 35.6%. 28.5% (38%) of respondents answered that the reason for not prescribing morphine is the absence of the national insurance coverage. 26.1% (38%) of respondents would prescribe if national insurance covers, and 63.6% (47%) of the respondents said they are determined to prescribe morphine whether or not the national insurance pays. Because our survey was conducted only 6 months after the policy change, many respondents may not have known the fact.

Conclusion: These results suggest that the recognition of morphine usage for ALS has changed, and it is very encouraging for us. We need an education system on the palliative care for ALS, because most of the respondents did not have enough experience of using these drugs for ALS. Therefore we have been organizing workshops on the palliative care for the ALS, twice so far. We hope all ALS patients in the world live peacefully until their end of life.

P9   PALLIATIVE APPROACH IN AMYOTROPHIC LATERAL SCLEROSIS: A POPULATION-BASED STUDY IN ITALY

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Keywords: ALS, palliative care, advance care planning

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder involving both upper and lower motor neurons; since the disease has a fatal course, patients' optimal management requires a palliative approach with the aim of improving quality of life (QoL) through the evaluation and treatment of clinical symptoms and respect of patients' decisional autonomy.

Aims: To evaluate the accessibility to Palliative Care Service (PCS) and the discussion rate of advanced directives in a population-based series of ALS patients' resident in Piemonte, northern Italy.

Methods and Results: In our study, we enrolled 187 patients with definite or probable ALS according to the revisited El Escorial criteria, diagnosed over a period of 3 years, from 1 January 2008 through 31 December 2010, in the province of Torino, Piemonte, Italy. Ninty-nine patients were male (mean age at onset: 66.2 years (SD 9.9)), 88 were female (67.1 years (SD 10.8)). In 128 cases, the onset was spinal, while in the remaining 59 onset was bulbar; 172 patients had a sporadic ALS, 15 had a familial form of the disease. At the final date of the follow-up (1 April 2013), 139 patients were dead. In our ALS population, 41 patients (21.9% of all cases and 29.5% of deceased patients) were referred to PCS. The discussion of end-of-life issues was performed with 101 (54.0%) patients: 16 opted for tracheostomy, 81 refused tracheostomy, and 4 were uncertain. Patients' advanced directives were followed in 90.6% of cases. We found no influence of gender, age at onset, ALS phenotype, marital status, and the use of NIPPV on patients' choice about tracheostomy, while patients performing PEG were significantly more favorable to undergo tracheostomy (p = 0.0005). Most patients died at home, as previously observed, but patients referring to PCS received more frequent palliative sedation (p < 0.0001). Finally, in our ALS population, 12.6% of patients underwent tracheostomy, in keeping with a previous report.

Conclusion: According to our study, palliative interventions could improve patients' QoL. In our opinion, it is necessary to make further efforts to increase the discussion rate of advance directives to comply with the personal autonomy of ALS patients.

P10　EXAMINING THE RELATIONSHIP OF BULBAR AND LIMB FUNCTION TO PATIENT REPORTED QUALITY OF LIFE: A MULTINATIONAL STUDY

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Keywords: quality of life, functional rating, multinational research

Background: The ALS-Specific Quality of Life Instrument-Revised (ALSSQOL-R) was validated using an American sample. We previously reported country-specific differences in ALSSQOL-R scores despite similarities in overall function as measured by the ALS Functional Rating Scale Revised (ALSFRSR).

Objectives: To examine the relationship of bulbar and physical functioning to the bulbar QOL and physical QOL subscores of the ALSSQOL-R, as judged by patients with ALS, in a multinational sample.

Methods: Three Canadian, one Swiss, one Israeli, and one Scottish ALS Centers recruited individuals with definite, probable, probable laboratory-supported, or possible ALS. American data were obtained from a previous multi-center validation study. The ALSSQOL-R was administered in subject's native language. ALSSQOL-R bulbar and physical subscores as judged by patients, ranging from 0 (worst QOL) to 10 (best QOL), were calculated. Bulbar function was determined by adding the ALSFRSR speech, salivation and swallowing items. Physical function was calculated by summing the ALSFRSR handwriting, eating utensils, turning in bed, dressing and hygiene, walking, and climbing stairs items. Pearson correlations and one-way analysis of variance were calculated with $P < 0.05$ as significant. The study was approved by the IRB/ethics committee of each institution.

Results: For the study, 396 American, 75 Canadian, 35 Swiss, 56 Israeli and 10 Scottish subjects were recruited. The five groups were similar in age, sex distribution, and disease duration. Bulbar and physical function scores were similar among the American, Canadian, Swiss, and Scottish samples; the Israeli sample was significantly poorer, $p < 0.05$. Correlations between function and patient-reported QOL in these two domains differed among the groups. In the American sample, there was a positive correlation of bulbar function and bulbar QOL ($r = 0.77$, $p < 0.05$), and between physical function and physical QOL ($r = 0.33$, $p < 0.05$). A similar pattern was found for the Scottish sample, but only between bulbar function and bulbar QOL, ($r = 0.73$, $p < 0.05$). In the Canadian and Israeli samples, there were no significant relationships of bulbar and physical function with their related QOL constructs. In the Swiss sample, a significant negative correlation of bulbar function and bulbar QOL was found ($r = -0.69$, $p < 0.05$).

Discussion and conclusion: The ALSSQOLR was developed and normalized on US residents. This study demonstrates possible country-specific or cultural differences in the relationship of function to QOL-related constructs. An alternative explanation is that differences in management of physical weakness (power lifts, power chairs, and hospital beds) and bulbar dysfunction (alternative communication devices and aggressive management of sialorrhea) may contribute to the impact of these factors on QOL. Caution is warranted when interpreting QOL measures in a population different from that in which the measures were validated. The factors which contribute to QOL in different countries, cultures, and disease-management systems merits further investigation.

P11　THE EFFECT OF A MULTIDISCIPLINARY CARE PROGRAM ON ALS PATIENTS SURVIVAL

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Keywords: multidisciplinary care, survival, ALS

Background: Multidisciplinary care units have become the preferred delivery of care model for amyotrophic lateral sclerosis (ALS) patients over the last two decades; however, the impact of this approach on overall survival is still under debate.

Objectives: We sought to evaluate the impact of multidisciplinary care on mortality in our ALS population.

Methods: Retrospective analysis of survival of ALS patients treated in a referral tertiary care hospital under a general neurology clinic (GNC) program and after the implementation of a multidisciplinary care program (MCP).

Results: We evaluated 417 patients, 84 patients were followed on a general neurology clinic and 334 on a multidisciplinary care program. On baseline, the GNC patients were 4 years younger (59 vs 63 years) and with a male predominance (66% vs 53%). The MCP patients had a greater proportion of bulbar onset disease (30% vs 21%), though it did not reach statistical significance ($p = 0.72$). MCP patients were more likely to receive riluzole (88% vs 50% $p < 0.01$), NIPPV (48% vs 33% p 0.016), and PEG (32% vs 10%). Kaplan–Meier analysis showed that median survival was increased by 3 months (logrank 10.08, $p = 0.001$). Cox modeling showed that the multidisciplinary unit program was associated with a reduced risk of death (HR: 0.66 IC 95 0.49–0.85). On the subgroup analysis, we found a greater effect on the bulbar onset patients (HR: 0.43 IC 95: 0.23–0.83), though it was also found to be protective on spinal onset patients (HR: 0.69 IC 95 0.49–0.98).

Conclusions: The implementation of a MCP on the management of ALS patients has a significant impact on mortality. Bulbar onset patients are especially prone to benefit from interventions associated with such program.

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P12   EFFECTS OF COGNITIVE BEHAVIOURAL THERAPY (CBT) IN PATIENTS WITH ALS AND THEIR PARTNERS; PRELIMINARY RESULTS

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Keywords: QoL, CBT, partners

Background: The progressive and disabling nature of ALS produce an enormous impact on both patient's and partner's quality of life (QoL). CBT may be of benefit to patients and their partners.

Objective: To study the effects of CBT in addition to usual care compared with usual care alone, on QoL in patients with ALS and their partners.

Methods: A multicentre, single-blinded, randomized controlled trial (1) was conducted. A CBT intervention was developed targeting QoL in patients and partners. ALS patients who met the inclusion criteria (score ≥ 8 on the Hospital Anxiety and Depression Scale (HADS)) and their partners were randomised to CBT or usual care (UC). CBT consists of individual psychological support in 5–10 sessions over a 16-week period. QoL was assessed using the Short Form 36 (SF-36) pre-intervention, post–intervention, and at 3- and 6-months follow-up. For each group differences in mean scores of SF-36 mental health subscales at pre-intervention and 6-months follow-up were analysed using paired-sample t-test: Social Functioning (SF), Role-Emotional (RE), Vitality (VT), and Mental Health (MH).

Results: Only 47% of ALS patients who gave informed consent met the inclusion criterion of an elevated HADS scores. This resulted in an inclusion of 15 ALS patients within 3 years. Ten couples were randomised to the CBT and five couples to the UC. The median of received CBT sessions was 3 (range: 0–10), 50% of the couples needed less than the pre-planned minimum of five sessions. In both groups, T3-data were missing, because patients died (CBT N = 1; UC N = 1) or were overburdened due to disease progression (CBT N = 1). For both groups patients' scores on SF, RE and MH subscales decreased, and VT increased. Partner's scores in the CBT increased on SF, RE and MH subscales, but decreased in VT, whereas partner's scores in the UC decreased in all subscales. However, all changes on the SF-36 subscales were not statistically significant.

Discussion and conclusion: The need for CBT in patients appeared to be low; on average three CBT sessions were sufficient to discuss their problems. Our preliminary results suggest that partners might benefit more than patients of a CBT-intervention targeting QoL. Future studies should focus more on needs of partners and other caregivers than on patients with ALS.

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P13   BREAKING THE NEWS IN AMYOTROPHIC LATERAL SCLEROSIS. ALS PATIENTS' REFLECTIONS ON THE TWO-TIERED APPROACH OF THE ALS CENTRE AMSTERDAM

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Keywords: breaking bad news, tailor-made patient care, supportive care

Background: According to standards in oncology, breaking bad news should be fine-tuned to patients' preferences, contain intelligible medical information, provide emotional support and offer a tailor-made treatment plan. To achieve these goals in ALS, neurologists of the tertiary ALS centre in Amsterdam see patients twice within 14 days to deliver the diagnosis and initiate the palliative care trajectory. However, this two-tiered approach to break the news has not yet been evaluated.

Objective: How do ALS patients reflect on the disclosure of the diagnosis and its consequences in a two-tiered appointment?

Methods: The first author performed non-participating observations of two-tiered appointments to break the diagnosis ALS, and subsequently interviewed patients in-depth about their experiences with this approach. Qualitative analysis consisted of open coding, followed by inductive analysis of all written material, observation reports and verbatim typed out interviews.

Results: Ten two-tiered appointments were observed and 21 ALS patients were interviewed in-depth about their views on this approach of breaking the news. All participants were native Dutchmen. They considered the sudden and full disclosure of suffering from an incurable and fatal disease as unavoidable and appropriate. The prospect of a short-term second appointment offered some structure for the period immediately following the devastating news. The time period between appointments left room for a first reorientation, allowing for the second appointment to be used to discuss various aspects of the diagnosis and its consequences in more detail. In addition, the second appointment was used to develop a plan for supportive management, adjusted to the needs and preferences of the patient.

Discussion: Our study shows that native Dutch ALS patients value full disclosure of the fatal diagnosis on the condition that professional follow-up is offered. The neurologist may play an important role in the transition of his patients to customized supportive and palliative care delivered by an ALS rehabilitation team. The approach of the ALS centre in Amsterdam may serve as a model for other – chronic – life-threatening diseases.

Conclusion: Dutch ALS patients value a two-tiered approach for breaking the bad news followed by a palliative care trajectory.

P14 DIFFICULTIES OF HOME CARE NURSES SUPPORTING INDIVIDUALS WITH AMYOTROPHIC LATERAL SCLEROSIS UNTIL END-OF-LIFE

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Keywords: end of life, home care nursing, ALS

Objectives: Amyotrophic lateral sclerosis (ALS) is a life-limiting, devastating disease. No effective treatments are currently available. Respiratory failure is the leading cause of death and sudden death is not rare. The median time of ALS from onset of symptoms to death is 23–48 months. Thus, health care providers need to support ALS patients with the consideration that death may occur at any time, while getting a positive attitude towards life from their patients. The purpose of this study is to clarify the illness situations of individuals with ALS at home and difficulties of home care nurses supporting such patients from the initial stage of home care nursing to death.

Method: The study design was retrospective and qualitative. Home care nurses from 11 home care agencies in four prefectures participated in this study. Semi-structured interviews were conducted to each home care nurse about their patient's circumstances from the beginning of home care nursing to the end-of-life, details of the type of support provided, and difficulties they experienced. Qualitative-inductive analysis method was used to extract categories and sub-categories. This study was approved by the institutional review board of the author's university.

Results: Data from 14 ALS cases were collected, Six cases died at home and eight died in hospital. The final respiratory management of patients was as follows: none for three cases, oxygen administration for one, non-invasive positive pressure ventilation (NPPV) introduced, but not used for four, intermittent NPPV for one, and tracheostomy positive pressure ventilation (TPPV) for five. Difficulties experienced by home care nurse consisted of five categories and 14 sub-categories. The category 'Support for decision-making of patients and families' consisted of three sub-categories, '*Difficulty to know the patient's thoughts*', '*Difficulty to communicate with patients*' and '*Difficulty having patients use medical treatments and social services*'. The category 'Selection of how to deliver care' consisted of three sub-categories including '*Difficult palliation*'. The category 'Insufficient home care support system' consisted of five sub-categories, '*Difficulty to get a home doctor*', '*Shortage of long-term facilities*', and three others. The category, 'Support for caregivers' consisted of the sub-category '*Difficulty to deal with family caregiver*'. The final category, 'Disagreement about how to support', consisted of two sub-categories, '*Dilemma*' and '*difficult assessment of approaching death*'.

Conclusion: To increase the possibility of peaceful death at home, it is important for home care nurses to develop the skills for assessment of approaching death, methods to deliver palliative care, and support for decision-making of patients and families. Also, it is suggested that improving the possibility of home visits by home doctors or giving the right of death diagnosis to home care nurses will provide better care for their patients.

P15 FRAIL TERMINALITY: HEALTH PROFESSIONALS' AND CARERS' DYNAMIC AND DIVERGING PERCEPTIONS OF CHRONICITY AND TERMINALITY IN ALS/MND

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Keywords: frail terminality, care work, coordination

Background: Several researchers have pointed out that ALS/MND resists a clear categorization as a chronic or terminal condition (1,2). In Norway, life sustaining technology is increasingly implemented in the hospitals, whereas a recent health reform (The 2012 Coordination Reform) reallocates further management to primary care and the home. The backdrop of this study is the unclear categorization of the ALS/MND diagnosis, and the need for good coordination in the complex division of labor in ALS/MND care.

Objectives: In this study we investigate how different actors in hospitals, primary care, and the home perceive and define ALS/MND as a chronic or terminal disease, and how this informs and motivates their work.

Methods: We have conducted 54 semi-structured interviews, lasting 1–2 hours, with carers at home, paid carers in primary care, and health professionals in hospitals and multidisciplinary teams, in Norway.

Results: The analysis demonstrates how the participants made continual assessments to whether a chronic or terminal framing should be used in perceiving day-to-day challenges. The actors involved in ALS/MND care emphasized chronic and terminal categorizations in subtly different ways, making for a potentially fragmented understanding of the challenge at hand. We suggest the concept *frail terminality* to describe such continual dynamics. ALS/MND is a fatal diagnosis. However, where the terminality is frail; it may be challenged and negotiated, as contingencies, choices, and interventions may make it less terminal. The indefinite time-scope of the prognosis may create an ongoing tension between enduring the situation in an unsustainable manner, appropriate for a terminal condition; simultaneously as the situation persists there is a need for more sustainable routines.

Discussion: The chronic-terminal unsettledness of ALS/MND complicates issues such as access to health services (hospice being one example), legal status (for instance regarding resuscitation), and attitudes among health professionals and carers. Awareness about how different stakeholders understand the challenges they encounter may facilitate a better coordination of care.

Conclusions: The concept of frail terminality may sensitise respective actors to articulate choices and dilemmas in ALS/MND care, allowing for more control in a potentially chaotic and overwhelming situation.

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P16   PLANNING AHEAD FOR PATIENTS WITH MOTOR NEURONE DISEASE

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Keywords: end of life, advance care planning

Objectives: To audit advance care planning (ACP) for MND patients under the care of a Regional MND Centre hospital multidisciplinary MND clinic, with reference to discussion about end-of-life care and documentation of the patient's wishes, and to assess sharing of information between the hospital and hospice, and/or community setting.

Method: Retrospective Case note review of MND deaths during a 2-year period.

Results: Data were collected from 38 deaths. 58% of patients died within 12 months of diagnosis. Place of death was hospital (47%), home (29%), care home (8%) and hospice (13%). 42% had received Non-Invasive Ventilation (NIV). There was evidence of ACP in 28 (74%) patients. This took the form of an Advance Decision to Refuse Treatment (18%), an advance statement about preferred place of care (13%), a 'Do not attempt resuscitation' order (53%) and medical letters or notes describing the end-of-life care plan (63%). ACP focused on the preferred place of care (14/28), resuscitation (12/28), ventilation (11/28), artificial feeding (10/28) and antibiotics (3/28). There were significant gaps in sharing of information between the hospital and community setting; of 13 patients with DNAR forms, only two patients had the DNAR documented in both sets of notes. Information sharing was most successful via copies of clinic letters (shared in 12/14). 56% of patients died in their preferred place of care. Where preferred place of care (PPC) was not achieved, 71% of patients died in hospital. Overall, end-of-life care for 76% of patients was deemed to have completely or partially complied with the patient's wishes.

Conclusion: A proactive approach to end-of-life planning by the multidisciplinary team led to evidence of planning ahead in 74% of patients. DNAR orders and compliance with refusal of ventilation and feeding was more successful than achieving PPC. Work is needed to strengthen ACP and coordination of care locally to reduce hospital deaths and improve patient care. Communication between care providers is the key to the delivery of high-quality end-of-life care for patients with MND.

P17   THE MEANING OF LOSS FOR PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS: IMPACT ON DECISION-MAKING IN CARE

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Keywords: loss, control, decision-making

Background: Few studies have investigated the meaning of loss for people with ALS, and no studies have explained how people with ALS engage with healthcare services as they adapt to loss. 'Biographical disruption' has been described in ALS, but little is known about how people with ALS construct loss and how their response to loss shapes how they engage with healthcare services.

Objectives: The aim of this study was to map key parameters of ALS service users' experience of healthcare services. The objective of the study was to identify how and why people with ALS interact with services.

Methods: Grounded theory method (1) was used to identify psychosocial processes which underpin how people with ALS engage with health and social care services. A diverse group of people with ALS (n = 34) were theoretically sampled from the Irish ALS population-based register. In-depth qualitative interviews were conducted with each participant in their homes. Data were analysed using open, axial and selective coding procedures (1). Codes formed concepts which in turn formed categories (variables) and the relationships between categories formed substantive theory to explain how people with ALS engage with services.

Results: Participants were living with insurmountable loss and never regained what they had already lost. The meaning of loss expanded across multiple dimensions including: loss of control; loss of identity; loss of the future; loss of parenthood; loss of hope; loss of participation; and loss of independence.

Losing control and fighting to remain in control comprised two central components in how participants interacted with service providers. The perception of loss of control prompted service users to exert control as they engaged with services. Participants expressed a desire to follow through with their own preferences for care and they engaged with services in line with their own perceptions of disability as opposed to those of service providers. Assistance from providers was more likely to be accepted and tolerated when perceived to be on participants' terms.

Discussion: We found that adaptation to loss in ALS is bidirectional: ALS service users navigate back and forth between resisting and accepting change. Living with ALS constitutes continuous loss for people with ALS, but they have the capacity to negotiate loss via complex appraisal strategies which include exerting control over and rendering control to service providers.

Conclusions: Service users' need to feel in control of health and social care services arises from their experiences of loss. Service providers should pay close attention to how people with ALS adapt to loss as they support them in the decision-making process about care.

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P18 WHERE AND HOW DO PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) DIE?

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Introduction: The final phase in patients with ALS has been the subject of few studies. It was our main objective to discover where and how patients die who are under observation from a Palliative Home Care Team (PHCT). Other, secondary objectives were to analyse the care burden, the main symptoms and the administered drugs during the tracking period.

Methods: Descriptive and retrospective study of patients with ALS followed up by PHCT (2001–2012), composed of a Multidisciplinary Unit of ALS. Analysis of variables through SPSS 15.0 and survival with Kaplan–Meier curves was carried out.

Results: Thirty-eight patients were followed up by the PHCT during the course of the study: 29 had died at the time the study was finalized; Mean age was 64 years (SD 12.5); 58% were females; Barthel index average 25; 89% had received previous instructions (33); 39% (15) presented bulbar ALS; 58% (22) spinal ALS; and 3% (1) familial ALS. In 45% (17) of the cases a gastrostomy was placed for feeding; 68% (26) used non-invasive ventilation (NIV); and 11% (4) used invasive ventilation. 82% (31) of the patients was taking riluzona at the start of the observation by PHCT and four patients died while taking the drug. The average observation period was 80 days. PHCT made an average of 14 visits (SD 13). The average number of hospitalizations per patient during the observation was 0.7. The median survival of patients since they started NIV was 8 months (CI 95% 2.4–13.6).

At the beginning of the observation by PHCT, the principal symptoms were pain (27%); sialorrhea (24%), dyspnea (21%); dysphagia (18%); and insomnia (5%). For 68% (19) of the patients who died, a subcutaneous route was needed during the last 24 hours. The most commonly administered drugs were morphine (68%); anticholinergic (39%); and midazolan (39%). 28% (8) needed palliative sedation because of the following reasons: 50% (4) dyspnea; 37% (3) rejection of invasive and non-invasive mechanical ventilation; and 12% (1) delirium. The drug that was applied in most cases was midazolan with a mean dose of 40 mg/day.

The percentage of the patients who died at home was 66% (19); 10% (3) in a hospice; and 24% (7) at a general hospital. The cause of death was respiratory failure 52% (15); respiratory infection 20% (6); and 10% (3) other causes, while 18% (5) of the patients died while asleep.

Conclusions: For patients that were observed by a PHCT frequent visits were necessary due to their diverse symptomatology. Most of the patients could die at home. The most common cause of death was respiratory failure that needed treatment with morphine in most of the cases and palliative sedation in 29% of the patients.

P19 INTERACTION OF PHYSICAL FUNCTION, QUALITY OF LIFE AND DEPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS: CHARACTERIZATION OF A LARGE PATIENT COHORT

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Background: Due to lack of any curative therapy of amyotrophic lateral sclerosis (ALS), symptomatic treatment and maintenance of quality of life (QoL) is very important.

Objectives: We aimed to characterize the affected domains of QoL in ALS patients and to identify impact factors on QoL and depression.

Methods: One hundred and fifty-nine ALS patients answered three standardized questionnaires (Beck depression inventory – II, SF-36 Health Survey questionnaire, and revised ALS functional rating scale). Multiple regression analysis and t-tests for independent samples were used to identify correlations between clinical features of ALS patients and depression/QoL scores. In addition, QoL data from ALS patients were compared to age-matched reference values representing the German normal population.

Results: QoL of ALS patients was reduced in nearly all SF-36-categories. Progression of physical impairment was positively correlated with depression, but hardly with deterioration of QoL. However, QoL was considerably influenced by depression, independent from physical impairment. Regarding distinct patient characteristics, advanced bulbar symptoms and increasing age were correlated with significantly worse QoL results regarding social functioning. Spinal onset patients suffered significantly more often from pain and physical limitations.

Conclusions: Depressive symptoms had a strong influence on QoL, hence their detection and treatment is of particular importance. Different domains of QoL are differently affected in subgroups of ALS patients. The knowledge about these differences can be valuable for both ALS caregivers and physicians.

P20 BULBAR SYMPTOMS AS PHYSICAL DETERMINANTS OF QUALITY OF LIFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease which can cause bulbar symptoms such as dysarthria, dysphagia and sialorrhoea. Previous work

has found quality of life (QOL) in ALS to be weakly related to physical dysfunction and psychosocial factors appeared more influential. However, bulbar symptoms may be very distressing to people with ALS and so it is important to explore how these symptoms may affect QOL.

Objective: To systematically review existing literature concerning the effects of dysarthria, dysphagia or sialorrhoea on the QOL of patients with ALS, in preparation for the Trajectories of Outcome in Neurological Conditions (TONiC) study, a multicentre national study of QOL in ALS.

Methods: MEDLINE (1990–2013), ScienceDirect (1990–2013), Ovid SP (1990–2013) and Compendex (1990–2013) were searched. Inclusion criteria stated studies must examine one of the three symptoms and have QOL as an outcome measure. The bibliographies of other studies which examined physical and psychological determinants of QOL in ALS were reviewed for additional references.

Results: For dysarthria, there were one non-blind experimental and one prospective study. In both studies, QOL was assessed in relation to communication support. Both concluded that there was a significant improvement in QOL with the introduction of communication support. Dysarthria was the only factor looked at in relation to QOL in the experimental study, but the prospective study also looked at non-invasive ventilation methods. For dysphagia, one survey was found on swallowing function. It concluded that apart from nutritional deficiencies created by dysphagia, there was also a reduction in QOL. For sialorrhoea, one double-blind randomized trial and five open-label prospective studies were found. The randomized trial showed no significant improvement in QOL after botulinum toxin treatment. From the five open-label studies, four looked at botulinum treatment and one looked at radiotherapy. All open-label studies concluded an effect on QOL.

Discussion and conclusion: The studies in this systematic review show variations in the methods used for measuring QOL, which impairs comparison between studies, and may have influenced findings. Each study has a small sample size which weakens the validity of their conclusions but as a group of nine studies, eight concluded that there was a relationship between QOL and bulbar symptoms. Physical, specifically bulbar, symptoms are associated with QOL in ALS. This highlights the possibility that psychosocial symptoms could be confounding physical symptoms when both are examined in combination. Further research is needed into the physical determinants of QOL, including looking at bulbar symptoms as they have been shown to have an impact on QOL in these initial studies.

P21   THE RELATIONSHIP BETWEEN BULBAR FUNCTION AND QUALITY OF LIFE IN PATIENTS WITH ALS

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Keywords: quality of life, bulbar function, multidisciplinary care

Background: The ALS Specific Quality of Life-Revised (ALSSQOLR) questionnaire measures six domains of QOL: Negative Emotion, Interaction with People and the Environ-

ment, Intimacy, Religiosity, Physical Symptoms, and Bulbar Function. Overall QOL in patients with ALS is not related to overall physical function. However, bulbar dysfunction produces unique challenges with regard to speech, swallowing, and secretion/mucus management, and the relationship between QOL and bulbar function has not been studied.

Objectives: To compare QOL in ALS patients with significant bulbar dysfunction to that of patients whose bulbar function is normal or minimally impaired.

Methods: An IRB-approved retrospective review of data collected in a multidisciplinary ALS clinic during a recent 24-month period was performed. The earliest visit of each patient was reviewed, excluding patients with significant cognitive impairment. Bulbar patients were classified as those who reported cumulative ALSFRSR bulbar sub-scores of 9 or less on items of speech, salivation and swallowing, corresponding to items 1–3 of the ALSFRSR. QOL was assessed with the ALSSQOLR, in which scores range from 0 (worst QOL) to 10 (best QOL) for average total QOL as well as the six domain sub-scores. Demographic data was also collected. Analysis of variance was performed comparing bulbar to non-bulbar groups on the dependent variables of average total ALSSQOLR score and the domain sub-scores.

Results: There were 100 patients: 48 men, 52 women, mean age 61 years, and 59% had bulbar. Men comprised 39% of the non-bulbar group and 54% of the bulbar group (p = NS). There was no difference in mean age between the non-bulbar (59 years) and bulbar (62 years) groups. Average disease duration was 55.2 months (SD 55.0) in the non-bulbar group and 42.8 months (SD 37.4) in the bulbar group (p = NS). Mean ALSFRSR score was higher in the non-bulbar than the bulbar group: 31.4, SD 8.8 vs. 21.4, SD 9.8 (p < 0.05). Bulbar patients reported a significantly lower average total QOL score (mean: 6.34) compared to non-bulbar patients (mean: 6.96), p < 0.05. There were no differences between the two groups in the six domain sub-scores, except for mean Bulbar QOL which was lower in the bulbar than the non-bulbar group: 4.32 vs. 8.01 (p < 0.001).

Discussion and conclusions: QOL in 5 of 6 domains is equal in ALS patients without and with significant bulbar dysfunction, but the bulbar QOL domain is lower in the bulbar group, resulting in a lower overall QOL. This was true even in a multidisciplinary clinic in which great attention is paid to symptom management and optimization of QOL. Further work needs to be done to determine how the impact of bulbar symptoms on ALS patients' QOL can best be addressed by health care teams.

P22   PSYCHOSOCIAL FACTORS AFFECTING QUALITY OF LIFE IN MOTOR NEURONE DISEASE: A SYSTEMATIC REVIEW OF THE LITERATURE

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Keywords: quality of life, psychosocial factors, TONiC study

Background: As motor neurone disease (MND/ALS) is progressive and fatal, supporting quality of life (QOL) is a

primary concern. Previous work has found QOL in MND/ALS to be weakly related to physical dysfunction, whereas some psychosocial factors appeared important.

Objective: To conduct a systematic literature review to determine psychosocial factors affecting QOL in MND/ALS, in preparation for the Trajectories of Outcome in Neurological Conditions (TONiC) study, a multicentre national study of QOL in MND/ALS.

Methods: Literature searches were conducted in Medline, Psychinfo, Cochrane Library, and the Amyotrophic Lateral Sclerosis and Frontotemporal Dementia journal to identify studies reporting relationships between psychosocial factors and QOL in people with MND/ALS.

Results: One hundred and three potential studies were retrieved after assessing the abstracts of publications identified through the searches. Of these, 75 were omitted on the basis of exclusion criteria such as reviews, case studies, diseases other than MND/ALS, outcomes other than QOL, psychosocial factors not included, and not published in English. This left 28 studies meeting the eligibility criteria. Five psychosocial factors were identified: These were social support; affective state (depression, anxiety, and hopelessness); religion/spirituality; coping strategies; and personality traits. Fifteen studies reported a positive relationship between social support and QOL. There were discrepancies among studies investigating affective state. Eight studies found that depression was negatively associated with QOL, whereas six found no significant relationship. Likewise, findings on anxiety were inconsistent. Four studies reported a negative association between anxiety and QOL while three found no relationship. Only two studies investigated hopelessness and in both it was negatively associated with QOL. While religion/spirituality was a positive factor in eight studies, one study disputed this. Coping strategies (two studies) and personality traits (two studies) received limited attention.

Discussion and conclusion: Social support has the strongest and most consistent evidence as a factor associated with QOL in MND/ALS. Discrepancies in the literature on affective state may be attributable to methodological considerations, such as differences in how QOL is conceptualised or measured. Fewer studies investigated anxiety than depression, despite several studies finding anxiety to be more strongly associated than depression with QOL. Anxiety may therefore represent an underestimated problem. Future research should establish its role. While the potential influence of religion/spirituality on QOL is strongly supported in the literature, one study found that patients infrequently identified it as a relevant factor. That study was conducted in a secularised country (Sweden). Future research should establish whether religion/spirituality is an important factor across different populations of MND/ALS patients. Coping strategies and personality traits warrant additional investigation. Of further note, all reviewed studies were observational. Interventional designs are required to investigate whether interventions targeting psychosocial factors positively affect QOL.

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P23 FUNCTIONING, FATIGUE AND PSYCHOSOCIAL FEATURES OF MND/ALS: ASSOCIATIONS CHANGE OVER TIME AND IMPACT ON PATIENT QUALITY OF LIFE

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Keywords: quality of life, longitudinal study, TONiC study

Objective: We conducted a longitudinal study of important physical and psychosocial features of amyotrophic lateral sclerosis (ALS/MND) and investigated their associations, change over time and impact on patient quality of life. The work forms part of the development of the Trajectories of Outcome in Neurological Conditions (TONiC) study, a national multicentre study of quality of life (WHOQoL-BREF) in neurological conditions including ALS/MND.

Methods: Patients were recruited from five hospitals in England (Liverpool, Oxford, Preston, Salford, and Sheffield), and completed questionnaires at baseline and at 6-month follow up.

Study variables were measured with disease-validated questionnaires for: functional capacity (ALSFRS-R); fatigue (MND-NFI); depression and anxiety (HADS); social withdrawal (MND-SWS); coping (MND-Cope); and WHOQoL-BREF.

The suitability of questionnaires for this population was confirmed using Rasch analysis, so deriving latent estimates suitable for parametric analysis. Consequently, data were analysed using linear regression modelling and t-tests.

Results: One hundred and seven patients recruited to a longitudinal study completed questionnaire packs 6 months apart.

Mean scores for depression increased over the course of the study ($p < 0.001$). Fatigue was strongly related to depression, anxiety, coping and social withdrawal at baseline ($p < 0.001$). Fatigue was not related to functional ability at baseline ($p = 0.33$), but was associated with reduced functional capacity at follow-up ($p = 0.003$).

Fatigue, social withdrawal and functional impairment substantially increased over the course of the study ($p < 0.001$). Mean scores for coping and anxiety remained stable throughout the study ($p > 0.05$).

WHOQoL-BREF at 6-month follow-up was associated with depression, anxiety fatigue, coping and social withdrawal at baseline, but not functional capacity. Fatigue and coping at baseline were the only significant predictors of WHOQoL-BREF at 6-month follow-up ($p < 0.05$).

Discussion and conclusion: Severity of depression appears to increase over the duration of the illness. Levels of depression were associated with fatigue, but did not predict WHOQoL-BREF at 6-month follow-up. Baseline fatigue and coping did predict QoL at follow-up.

This study provides longitudinal evidence demonstrating that high fatigue and poor ability to cope lead to reduced WHOQoL-BREF for patients with ALS/MND.

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RIGHTSLINK

P24   PHYSICAL THERAPY AND EXERCISES TO PATIENTS WITH ALS

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Background: Danish physical therapists in the ALS teams are responsible for exercise guidance, evaluating needs for assistive devices, and chest physical therapy. Until now there has been no consensus among physical therapists, in Denmark, about which treatment to use and when to use it. Only very few articles, describe in detail the treatment given by physical therapist to patients with ALS.

Objectives: The aim of this study was to investigate which treatment is used, what is the evidence for the treatment, what treatment/instructions do the patient think is important, and based on that create a national clinical guideline in Denmark.

Methods: Fourteen hospitals in Denmark diagnose and treat patients with ALS. Thirteen have an ALS team, with a physical therapist. All 13 teams are included in the study.

The study is divided into three steps.

Step 1: Involvement of the physical therapists: (a) Answering a semistructured questionaire about their treatment strategies to patients with ALS; (b) Participation in a workshop, with focus on discussion of evidence, treatment strategies and creating consensus about the physiotherapeutic treatment to patients with ALS in the hospitals in Denmark; and (c) Qualifying the clinical guideline by testing it in clinical practice.
Step 2: Involvement of the literature: (a) A systematic search in the databases Pubmed, Cinahl, Cochrane and Pedro and (b) Evidence extracted.
Step 3: Involvement of the patients: (a) A focus group interview to assess what the patients think is important in the physical therapy treatment/instruction and (b) The clinical guideline is sent in consultation.

In the process there has been contact to The Danish Institute for Clinical Guidelines.

Results: Both the physical therapists, the patients and the literature agree that the physical therapist has an important role in guidance regarding respiration, exercise and assistive devices. Five papers included data about exercise and 12 about chest physical therapy. A national clinical guideline has been made and contains nine recommendations on exercise and 12 on chest physical therapy based on evidence level B–D.

Discussion and conclusion: A clinical guideline has been made and builds on best practice experience from the Danish ALS physical therapist, the patient view and evidence from the literature. The evidence level is low because there is lack of resource regarding exercise and chest physical therapy to patients with ALS, and there is a high dropout rate in the studies due to the nature of the disease. By standardizing physical therapy to ALS patients in Denmark, treatment quality is ensured and resources are made possible.

P25   EFFECTS OF ENDURANCE TRAINING ON QUALITY OF LIFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: endurance, quality of life, amyotrophic lateral sclerosis

Background: The control of fatigue can improve function and Quality of Life (QoL) in patient with progressive illness, reducing physical, psychological and spiritual distress. The role of exercise to reduce fatigue in people with ALS is controversial. However, recent studies promoting aerobic exercise in ALS patients as a source of well being on psychology of patients have been published (1,2).

Objectives: The aims of this study are to determine proper loads of therapeutic programs suited for each patient and to determine the effects of endurance training on QoL.

Methods: Twenty consecutive ALS patients (10 male and 10 female) have been recruited for this study and randomized in two groups: Control Group (CG) in which patients receive usual care and cicloergometer without increase in time training and load and Experimental Group (EG) in which patients received usual care and cicloergometer aerobic training in the stronger pair of limbs. The aerobic training was repeated thrice a week for 4 weeks. All patients underwent the following evaluations, at baseline and at the end of treatment: Cicloergometer incremental Test, ALS Funtional Rating Scale-revised (ALSFRS-R), Hospital Anxiety and Depression Scale (HADS), ALS Quality of Life-40 items (ALSAQ-40), Mini Mental State Examination (MMSE), and Fatigue Brief Inventory-Italian version (3). After every treatment patient underwent Numeric Pain Rating Scale (NPRS), Numeric Rating Fatigue Scale (NFRS), BORG Scale, and Fatigue Brief Inventory-Italian version.

Results: Mean age at evaluation was 60.7 ($\pm$12.4) years. Mean ALSFRS-R score was 33.38 ($\pm$5.78). Abnormal value of ALSAQ-40 were detected in 30% of patients (cut-off > 69), HADS was impaired in 55% of patients (cut-off > 8). A relationship between the mean score of Brief Fatigue Inventory and Anxiety ($\chi^2 = 28$), Depression ($\chi^2 = 28$), QoL ($\chi^2 = 42$), and MMSE ($\chi^2 = 28$) were found (p-value < 0.05). At baseline Brief Fatigue Inventory mean score was 26.7 ($\pm$ 24.68), at T2 was 34.8 ($\pm$ 24.72). Differences in the Brief Fatigue Inventory between the CG and the EG was detected (t = 2.58) (p-value < 0.05).

Discussion: Preliminary data showed positive effect of training on ALS patients fatigue and QoL. However, further analysis is needed to better clarify these observations.

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P26 AN EVALUATION OF THE EFFECT OF CORTICOSTEROID INJECTION ON SHOULDER PAIN AND SLEEP QUALITY IN PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: shoulder pain, steroid injection, sleep

Background: Shoulder pain and discomfort is a common feature of Motor Neuron Disease (MND) (1). Particular problems with shoulder pain arise for MND patients at night (2). Corticosteroid injection therapy (CIT) provides an effective treatment for shoulder pain (3), however, there is no evidence regarding its efficacy in MND patients specifically.

Objectives: To determine the effect of CIT on shoulder pain and sleep quality in patients with MND.

Methods: Patients with shoulder pain were identified at MND multidisciplinary clinic and suitability for treatment with CIT was determined. History of shoulder pain, intensity measured using numerical rating scales (NRS) and sleep quality using a Modified Pittsburgh Sleep Index were recorded. Patients were contacted by telephone 2 weeks and 2 months post CIT to reassess pain, sleep quality and global perceived change. Data were entered into Microsoft excel and exported to STATA 12 for analysis. Summary statistics were derived and change over the three time points was assessed using one-way ANOVA.

Results: Fourteen patients (F = 9) with shoulder pain, with average age of 63 ± 9.8 years, were treated with CIT. On reassessment at 2 weeks, 93% reported an improvement on the global perceived change and 88% reported improvement at 2 months. Two patients reported complete recovery of shoulder pain at 2 weeks and 2 months. Two patients were unable to complete 2-month follow-up. Average daily pain on NRS at baseline was 4.9 ± 2.6, at 2 weeks was 2.3 ± 1.7 and at 2 months was 2.1 ± 1.7 indicating a significant change post treatment (p = 0.003). Night pain also reduced, from 5.6 ± 3.3 to 2.5 ± 2.5 at 2 weeks and 1.9 ± 2.5 at 2 months (p = 0.006). The number of nights with sleep disturbance due to shoulder pain reduced significantly from 6.2 ± 1.5, to 4.8 ± 3.1 and to 3.8 ± 3.3 (p = 0.026).

Discussion and conclusion: CIT was found to significantly reduce shoulder pain, night pain and the number of nights with sleep disturbance due to shoulder pain in MND patients. This effect was seen both at 2 weeks post CIT and was maintained at 2 months post CIT. Therefore this finding provides strong evidence that this treatment should be offered to MND patients with shoulder pain and reduced sleep quality.

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P27 RELATIONSHIP BETWEEN QUALITY OF LIFE AND RESPIRATORY ASPECTS, DIAGNOSIS TIME AND FUNCTIONALITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: quality of life, respiratory aspects, functionality

Introduction: Weakness and muscle atrophy, spasticity, fasciculations, dysarthria, dysphagia and respiratory failure are symptoms of ALS patients. The clinical picture of such individuals can influence the quality of life (QOL), defined according to the World Health Organization as how individuals understand their position in life, culture context, value systems and the relation established with the environment they live (1–3).

Objective: To correlate the QOL of ALS patients with respiratory aspects, diagnosis time, functionality.

Method: Cross-sectional study performed in the department of research on neuromuscular diseases, Federal University of São Paulo (UNIFES), Brazil. Based on the El Escorial criteria, able to answer questionnaires and conduct pulmonary function tests, we included subjects with confirmed diagnosis of ALS. There were 22 participants with average age of 54.59 ± 7.01: 11 (50%) men; 11 (50%) women. For the QOL assessment, participants answered the SF-36 and WHOQOL questionnaire. Tests applied are forced vital capacity (FVC); maximal inspiratory pressure (MIP); maximal expiratory pressure (MEP); and peak cough flow (PCF). From the medical records, we collected diagnosis time, onset of symptoms and use of non-invasive ventilation; moreover, using ALSFRS scale, we investigated the functionality. We also used the Pearson test, with significance level of p < 0.05.

Results: The WHOQOL physical domain correlates with MEP (r = 0.042/p = 0.049), PCF (r = 0.516/p = 0.014) and ALSFRS scale (r = 0.477/p = 0.025); WHOQOL social relationships domain, with ALSFRS scale (r = 0.452/p = 0.035). The SF-36 physical functioning domain positively correlated with MEP (r = 0.463/p = 0.030), PCF (r = 0.563/p = 0.006) and ALSFRS score (r = 0.593/p = 0.004). Moreover, the SF-36 physical limitations domain correlated with MEP (r = 0.482/p = 0.023) and PCF (r = 0.598/p = 0.003); SF-36 general health status domain, correlated with ALSFRS scale (r = 0.451/p = 0.035). The SF-36 mental health domain showed positive correlation with diagnosis time (r = 0.474/p = 0.026).

Discussion: In ALS, FVC is an indication of respiratory impairment; however, ALS did not correlate with QOL, and only MEP and PCF values can affect on QOL. From the findings herein, functionality is enormously important for the QOL of ALS patients. The mental health domain correlated with diagnosis time, that is the longer the disease period the

better the QOL in the mental health domain. This result may be related with the changes and acceptances imposed during the course of the disease.

Conclusion: The QOL, physical functioning-related domains and general health correlate with MEP, PCF and functionality; also, mental health domain correlates with diagnosis time.

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P28 DISABILITY, ASSISTANCE AND MOBILITY AIDS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: disability, ALSFRS-R, quality of life

Background: In patients with ALS, functioning may be described according to the assistance and mobility aids that are required. The goal of this study is to assess the validity of a disability classification system that is based on the ALSFRS-R and includes four levels of disability: mild, moderate, severe and extreme.

Methods: During an initial evaluation, 140 patients with ALS (mean age 57.3 years, SD 12.4; 59.2% male) were classified according to El Escorial criteria, the ALSFRS-R, the rate of progression of the disease and the number of muscles with severe weakness. The rate of progression of the disease was defined as the ratio of the actual ALSFRS-R/time from onset (months). For the neck flexion time, patients were asked to hold their heads flexed as long as possible (seconds). For the counting test, patients were asked to take a deep breath and count up until a new breath was needed (last number registered).

All patients were contacted 6 months later. Just 101 answered the ALSFRS-R and other questions related to assistance. Assistance was measured with an ordinal scale (0–8) based on the presence/absence (0/1) of nursery support, BPAP/CPAP, supplementary oxygen, wheelchair, dippers, gastrostomy, communication devices and need for other persons for transferences inside the house.

The levels of disability were established by taking one representative activity from the three domains of the ALSFRS-R that were defined by factor analysis (walking, cutting food and swallowing). Each of these was dichotomized as dependent (ALSFRS-R of 0, 1 or 2) or independent (3 or 4). Mild disability was defined as independence in all of the activities, moderate as dependence in one and independence in the other two, severe as dependence in two activities and independence in one. Extreme disability was defined as dependence in all three activities.

Results: A correlation was found between the disability scale and the rate of progression ($r = 0.43$, $p < 0.001$), the number of severely weak muscles ($r = 0.33$, $p < 0.001$), the neck flexion time ($r = -0.48$, $p = 0.004$) and the counting test ($r = -0.48$, $p = 0.001$). A correlation with the ordinal scale of assistance was found ($r = 0.77$, $p < 0.001$), especially with the number of persons needed for transferences ($r = 0.6$, $p = 0.001$).

Nursery care was needed in 0%; 0%; 23.1%; and 70.1% for the four levels of disability. The proportion of wheelchair-bound patients was 14.3%; 25%; 58.3%; and 94.1% (chi square $p < 0.001$). The need for other persons for transferences was 0%; 14%; 28%; and 57.1%. Non-significant correlation was found with BPAP/CPAP or supplementary oxygen.

Conclusion: This disability classification system is simple and easy to use in everyday practice. It is also useful as a guide for treatment and rehabilitation and as a reference for an easier communication between those involved in the care of patients.

P29 UROLOGICAL MANAGEMENT IN AMYOTROPHIC LATERAL SCLEROSIS/MOTOR NEURON DISEASE (ALS/MND): SUBRAPUBIC CATHETERS CAN IMPROVE QUALITY OF LIFE BUT ARE UNDERUTILIZED

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Keywords: urinary tract, suprapubic tract, diaphragm pacing

Background: Suprapubic catheters are standard therapy to treat neurogenic bladder in neuromuscular diseases such as spinal cord injury and multiple sclerosis. Urology literature also supports the use of indwelling catheters for immobility and end-of-life comfort. Review of the literature shows minimal information for use in ALS/MND. In other tetraplegics use of a suprapubic catheter in patients led to less urological complications and a better quality of life scores.

Objectives: Analyze the outcomes of ALS/MND patients who received suprapubic catheters.

Methods: Subgroup retrospective analysis of a prospective database at a single site of all patients who had suprapubic catheter placement was carried out.

Results: Six patients had suprapubic catheters placed with three patients having simultaneous diaphragm pacing (DP), suprapubic catheters and feeding tubes all placed at the same surgical encounter. Three are males. Average age is 56 with range of 35–69 years old. All were wheelchair bound requiring full lifts to transfer. There were no surgical complications. There has been no cross-contamination or infections between the surgical sites. There have been no significant long-term complications. One patient reports having had three urinary tract infections in 5 months. All patients report benefits of catheter outweigh the negatives. Benefits reported are increase in freedom and eases care. Patient's primary caregivers also

report same benefits of suprapubic. No patients have returned to their pre-suprapubic catheter regimen and all state they would have done this sooner.

Discussion: Indwelling suprapubic catheters have been used in other neuromuscular diseases as standard therapy. ALS/MND patients may not experience neurogenic bladders or as have as much spasticity, but they do have the same immobility issues. For patients who require lifts to transfer from the wheelchair to a commode makes leaving the house difficult. Physiologically, immobile women require significant more care. We have had patients who decrease their oral intake and allow themselves to be slightly dehydrated to avoid having to urinate. The use of suprapubic would obviate these difficulties. Having a surgical team which includes an urologist available to work together can help simplify bladder issues for patients by having several procedures done simultaneously. The major risk of a suprapubic catheter is infection and the catheter requires routine maintenance with monthly changes.

Conclusion: Suprapubic catheters are low-risk surgical procedures that can be done outpatient. For patients undergoing DP, the placement of a suprapubic is a short extension of surgical time. Benefits outweigh the risks and should be discussed in the appropriate clinical conditions.

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P30   HOW MUCH IS THE OPTIMAL INITIAL DOSE OF MORPHINE FOR ALS PATIENTS?

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Keywords: palliative care, morphine, initial dosage

Background: We started to use morphine to palliate dyspnea in ALS patients in 2005. At that time, morphine use was not approved by national health insurance in Japan. In September 2011, at last national health insurance gave permission to use morphine on ALS patients. Since then, more and more neurologists started to use morphine. However, administration protocol, optimal dosage, effectiveness and side effects in Japanese ALS patients are not yet clarified. Since morphine has been used on ALS more than 40 years ago, it is very difficult to find out good evidence for the optimal initial dose of morphine for ALS.

Objectives: To clarify administration protocol, optimal dosage, effectiveness and side effects in Japanese ALS patients.

Methods: Thirty-three ALS patients whom morphine was used to palliate dyspnea were analyzed. We used 2.5 mg (1.25 mg in case $PaCO2 > 60$ Torr) of morphine chloride PO or IV as an initial dose. Dosage was increased until relief of dyspnea was achieved (maximum dose 10 mg), and administration was repeated based on patients needs.

Results: Morphine was used in 33 ALS patients between December 2007 and April 2012 (age: 43–93 years, average disease duration 35.6 month). Although initial dose was 2.5 mg in 47%, 1.25 mg in 27%, and 5mg in 10% of patients, initial effective dose was 2.5 mg in 40%, 5 mg in 37%, and 1.25 mg in 13% of patients. Maintenance dose was 30–60 mg. Final dosage at the time of death was 5–230 mg (average 56.1 mg). Morphine was effective in palliation of dyspnea in all patients. PCO2 was not increased after morphine administration in 28 patients (85%) except for the patients whose remaining days were less than several days. Side effects were constipation (31 cases), sleepiness (2 cases), and drowsiness (2 cases). Respiratory distress was observed in only one case.

Discussion and conclusions: Morphine is very effective and can be used safely in Japanese ALS patients. An initial dose should be 1.25–2.5mg depend on PaCO2. Maintenance dose was much lower compared to cancer patients. Respiratory distress was observed in only one case. We are afraid that even now some Japanese neurologists are reluctant to use morphine. A survey conducted on physician treating ALS in 2012 showed that only 30% of Japanese neurologists use morphine on ALS. We assume the reasons are (1) fear to cause respiratory distress; (2) prejudice that morphine is only used for pain; and (3) not familiar with opioids use. Dyspnea is one of the most severe sufferings in ALS patients and at the moment morphine is the best choice. All ALS patients must be provided with proper palliative care. We must keep trying to make more Japanese neurologists accept the importance and usefulness of morphine on ALS patients and make a chance to learn about palliative care for ALS.

P31   VOICE BANKING AND VOICE RECONSTRUCTION FOR MND PATIENTS

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Keywords: voice output communication aids, voice reconstruction, speech synthesis

When the speech of an individual becomes unintelligible due to a degenerative disease such as motor neurone disease (MND), a voice output communication aid (VOCA) can be used. To fully replace all functions of speech communication – communication of information, maintenance of social relationships and displaying identity – the voice must be intelligible, natural-sounding and retain the vocal identity of the speaker. Attempts have been made to capture the voice before it is lost, using a process known as voice banking. But, for patients with MND, the speech deterioration frequently coincides or quickly follows diagnosis.

Using model-based speech synthesis, it is now possible to retain the vocal identity of the patient with minimal data recordings and even deteriorating speech. The power of this approach is that it takes advantage of the speech already collected from several voice donors so that only the essential parts of one individual's voice must be recorded.

Ideally, we record the patient's voice early on in the disease and create a synthetic version of his voice for use if required at a later date when speech may become affected. However, if the patient's voice has begun to deteriorate at the time of the recording, a voice repair technique can be applied

in order to compensate for the disordered characteristics found in the patient's speech.

We present here an ongoing project for voice banking and voice reconstruction based on this technology.

P32   FINDING IN THE INTELLIGIBILITY SCALE OF SPEECH RELATED TO MYOELASTIC-AERODYNAMIC MECHANISMS OF PATIENTS WITH MOTOR NEURONE DISEASES

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Keywords: speech intelligibility, fatigue, speech

Background: The loss of oral communication has been broadly argued as a strong indicator of a decrease in the quality of life in patients with MND. One of the factors that cause the change in the intelligibility of speech is the denervation of the tongue muscle and the limitation of expiratory flow.

Objective: To analyze the relation among the intelligibility of speech scale, the peak cough flow and tongue fatigability.

Methods: The assessment was carried with 37 patients, 24 (64.8%) of them had amyotrophic lateral sclerosis (ALS) and 13 (35.2%) had progressive bulbar paralysis (PBP). The speech therapist evaluation included tongue fatigability in lateral movements, tip elevation and back elevation after verbal commands, intelligibility scale of speech, and peak cough flow (PCF).

Results: The first (1) degree on the intelligibility scale refers to vocal communication and the tenth degree (10) to normal speech. Patients with ALS who showed 1 degree did not have any voluntary tongue movement and the average PCF was 93.3 l/minute (l/min); 2 degree ALS: average PCF of 115 l/min and tongue fatigue indication up to three isotonic movements; 5 degree ALS: average PCF of 145 l/min and fatigue after five movements; 7 degree (ALS): average PCF of 289 l/min and fatigue after 5–12 lateral movements, 3–8 movements of tip elevation, 5–18 movements of back elevation; 8–10 degree ALS: average PCF of 322 l/min fatigue after 4–20 lateral movements, 7–10 tip elevation movements and no fatigue after 20 back tongue elevation. Patients with PBP with 1 degree did not have any voluntary movement of the tongue and the average PCF was 50 l/min; 2 degree PBP: average PCF of 217 l/min and tongue fatigue that varied between absence of contraction and fatigue after three movements; 5 degree PBP: average PCF of 325 l/min and tongue fatigue after 3–8 lateral movements, four tip elevation movement and eight back elevation movements; 7 degree PBP: average PCF of 225 l/min and tongue fatigue after 5–20 lateral movements, 3–10 tip elevation movements and 15–16 back elevation movements.

Discussion: There is a relationship between aerodynamics and myoelastics mechanisms speech. To know and to respect the limits of muscle fatigability is one of the main challenges of rehabilitation work. The speech therapist should include therapeutics strategies of breathing and myofunctional therapy, with caution in prescribing the type and amount of exercises in patients with MND.

Conclusion: It was observed that the lower the speech intelligibility, the lower is the peak cough flow and the symptoms of fatigue will be evident sooner, showed by the reduction in the range of motion, the worsening of the quality of movement or respiratory distress associated with the task requested.

P33 A SURVEY OF POWER WHEELCHAIR USERS WITH ALS/MND; CHANGING NEEDS OVER TIME

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Keywords: power wheelchair, quality of life, survey

Background: Recent publication by Ward *et al.* in 2010 began the process toward developing "must haves" on a power wheelchair for persons with ALS/MND to meet their long-term needs. One other article exists which also looks at powered wheelchair use in ALS/MND from 2001. Rapid progression of the disease means the power wheelchair must be flexible for comfort and function.

Objectives: To survey persons with ALS/MND at 1 month and 6 months after receiving power wheelchairs to determine what is required for long-term effective use, comfort and function.

Methods: Thirty-three-question survey and Psychosocial Impact of Assistive Devices Scale (PIADS) was sent 1 month after getting a new power wheelchair, follow-up survey sent at 6 months. The survey addressed satisfaction, feature use, comfort and function with the power wheelchairs.

Results: Based on satisfaction and feature use survey results, at 1 month, 56% of users are performing tilt, recline and elevating legs at least five times a day, and at 6 months 50% are performing these tasks. For users at 1 month, 72% are still pleased with their choice of cushion, headrest and power features, and this percentage drops only slightly at 6 months. Their quality of life increased and pain decreased at 1 month and 6 months. According to the PIADS, the power wheelchair gave users increased ability to participate and sense of competence.

Discussion: Power wheelchair use is a stage most people with ALS go through during the disease process, and it is important that Physicians, Nurses and Allied Health Clinicians are aware of the need for long-term flexibility in the power wheelchairs, as well as what users of these chairs say about their function and comfort requirements.

Conclusion: This survey has important results for the ALS/MND population, and is the first of its kind to assess power wheelchair users at 1 month and 6 months after getting their chairs. Our results demonstrate the usage, psychosocial issues, function and quality of life impact that the power wheelchair has on the life of someone with ALS/MND.

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P34 EYE-TRACKING COMPUTER SYSTEM UTILIZATION BY PATIENTS WITH ADVANCED AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: eye-tracking system, locked-in, alternative augmentative communication

Background: Most ALS patients in the advanced stages of the disease become locked-in (LIS). This is a severe clinical condition characterized by quadriplegia and anarthria, with preservation of consciousness and eye movements. Taking advantage of preserved eye blinking and movements, LIS patients can now use an eye-tracking computer system (ETCS) as an interface for augmentative/alternative communication (AAC). ETCS allows an easy access to communication, internet navigation, webmail, social networks and even domotics. A few studies have suggested that the device has a high rate of acceptance in ALS, with a positive impact on quality-of-life.

Objectives: We performed a telephone interview to the ALS LIS patients, living in Sicily, which had received an ECTS device between January 2011 and June 2012. We explored the clinical and demographic variables that are likely to affect its utilization, the level of acceptance and the impact of this device on the patient's ability to be in contact with the outer world.

Methods: Twenty-seven consecutive non-demented ALS patients at LIS stage were enrolled. Mean age at interview was $55.1 + 11.6$ years, with a median education of 13 years (8–13). Among ALS patients, onset was spinal in 23 and bulbar in four, with an M/F ratio of two. All patients were previously trained for ETCS use. A telephone interview was made to each patient. Answers were provided in a subsequent phone call with the help of the caregiver. The interview included items about demographic and clinical variables, and factors affecting the daily ETCS utilization.

Results: At the time of interview, patients were with the ETCS device for a mean period of $15.8 + 11$ months, with a daily utilization of $359 + 266$ minutes. ETCS users were young ALS patients and with high education when compared to the whole population of ALS. Patients mostly used ETCS to communicate with caregiver/relatives and to navigate social networks/internet. 33.6% of LIS patients (all were ALS) had a rather low daily utilization of the ETCS device (ie less than two hours); most frequent reported causes were early eye gaze, fatigue and oculomotor dysfunction.

Discussion and conclusion: ETCS is a useful AAC device and most LIS patients can use it with a good performance for a wide range of purposes, including internet navigation. However, our study suggests that the device is mostly requested,

and fruitfully used, by relatively young patients with a rather high education. The development of oculomotor impairment may limit the ETCS correct use.

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P35 SATISFACTION OF ALS CLIENTS AND CAREGIVERS WITH TRAINING ON USE OF AN EYE-GAZE SYSTEM IN A FACILITY SETTING

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Keywords: eye tracking system, communication, satisfaction

Background: Due to the progressive nature of ALS, some clients eventually reach a locked-in state making communication a major challenge. This is particularly true for clients who choose invasive ventilation as an intervention. Eye-gaze is often the only available access method to high-tech AAC for this population. The Vancouver Coastal Health ALS Centre OT and SLP have been offering support and training to clients, caregivers and families on how to use eye-gaze systems.

Objective: To evaluate satisfaction levels of clients, families and healthcare providers with training and use of an eye-gaze system in a facility setting.

Method: ERICA eye-gaze systems were allocated to three ventilator dependent ALS clients living in one care facility over a 4 year period. Training sessions were provided to clients, facility staff and caregivers. Limited technical support was offered over the phone and by email. A satisfaction questionnaire, including 7–8 items on a 5-point Likert Scale and five open-ended questions, was distributed to clients and caregivers after receiving the device. Five semi-directed interviews were conducted, and data were obtained from three clients, 15 healthcare providers and two family members. Results from the questionnaires were collated and descriptively analyzed. Interviews were recorded, transcribed and qualitatively analyzed.

Results: ALS clients, their families, and the healthcare providers generally expressed a high level of satisfaction with the use of the eye-gaze system; higher in those with more family support and reported higher motivation to use the internet. Clients with limited family support and limited computer skills were more dependent on facility staff for set up and opportunities to use the device. ALS clients expressed a higher satisfaction with the eye-gaze training provided compared to the caregivers.

Discussion: While the sample size was small, ALS clients and their caregivers identified the need for more caregiver training to increase proficiency with the eye-gaze system. The greater satisfaction reported by clients with more family support, may be due to increased opportunities for practice. Barriers to successful implementation of eye-gaze use in a facility setting may include staff accessibility and turnover, limited family participation, and eye-gaze software difficulties and set up concerns.

Conclusion: Caregiver comfort with technology and sufficient training on device set-up are keys to successful eye-gaze use for ALS clients. These factors appear to be essential in maintaining an enhanced quality of life for the users. Future plans could include comparing competitor eye-gaze system use, and comparing caregiver satisfaction ratings between facility, home and acute care settings.

P36 VIDEOENDOSCOPIC EVALUATION OF SWALLOWING (VEES) IN PATIENTS WITH MOTOR NEURON DISEASE/AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: videoendoscopic evaluation of swallowing, dysphagia, deglutition

Background: Dysphagia is a common, serious symptom in motor neuron disease (MND). In primary lateral sclerosis (PLS), degeneration of upper motor neurons can result in pseudo-bulbar paralysis. In amyotrophic lateral sclerosis (ALS) also lower motor neurons in the brainstem are affected: at disease onset in about 30%, in later stages in nearly all patients. Monitoring deglutition is crucial to avoid malnutrition, weight loss, aspiration, and to timely implement diet changes, adaptive strategies, or tube feeding. Videoendoscopic examination of swallowing (VFES) noninvasively visualizes pharyngeal dysphagia and effectiveness of compensatory maneuvers (1).

Objectives: To retrospectively report on VFES and therapeutic consequences in consecutive MND patients.

Methods: Prior to VEES, a speech and language therapist examined each patient clinically and documented the current diet by the functional oral intake scale (FOIS) (2). VEES was indicated to evaluate whether deglutition was safe, swallowing strategies and diet appropriate. For assessment of phyaryngeal swallowing, we used the Penetration Aspiration Scale (PAS) (3) and noted if residues were present.

Results: Between April 2007 and March 2013, 45 consecutive MND patients underwent VEES. There were 31 men and 14 women (mean age: 66 and 64 years, range: 31–85 years); three men and one women were diagnosed PLS. ALS was characterized by bulbar-, upper-, and lower-limb-onset in 10, 13, and 18 patients, respectively. Mean disease duration at first VEES was 4 years (range: 1–17 years). Nine patients had a gastric tube (PEG). Eleven patients needed multiple VEES evaluations because of disease progression. In total 64 VEES were performed; only one was stopped due to excessive choking. Pharyngeal deglutition was preserved in most assessments, but in 23 of those 49 VESS with PAS 1, relevant residues were detected. After VEES, we

recommended modification of diet and insertion of PEG in 19 and 9 patients, respectively. According to 39 VEES examinations, adequate feeding and swallowing strategies were demonstrated and patients as well as their carers could be reassured.

Discussion and conclusion: VEES is a non-invasive, well-tolerated method to monitor deglutition and security of nutrition in all stages of MND. As it provides relevant information for the management of dysphagia, nutritional surveillance, dietary counselling, timing of enteral nutrition, and can improve insight of patients and carers in swallowing problems, it should become an integral part of diagnosis in multiprofessional ALS centers.

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P37 PALATAL PLATE IMPLANTATION FOR THE TREATMENT OF DYSARTHRIA AND SEVERE HYPERNASALITY IN ALS PATIENTS

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Keywords: ALS, palatal plate, dysarthria

Background: Dysarthria in ALS is typically characterized by muscle weakness and hyposthenia of buccofacial districts, causing inaccurate production of phonemes sometimes associated to dysphonia and hypernasality.

Objectives: To evaluate if a palatal plate, stiffing the soft palate, can improve dysarthria and severe hypernasality of a patient with bulbar ALS, increasing the intelligibility of speech.

Case report: A 60-year old woman with a one-year history of bulbar onset ALS was referred for a phoniatrics assessment. She had ALS-FRS-R score of 41, with a speech score of 2. Clinical and instrumental examination showed signs of mild dysarthria, severe hypomobility of velum palatinum, reduced strength and precision of exercises of tongue, mouth and face, normotrophic tongue, absence of dysphagia. She underwent rhinomanometry and acoustic analysis of voice. The patient was adapted to a palatal plate on measure after dental impression; this device has a retainer for removable prostheses located in the dorso-palatal area of the mouth. The acrylic plates elevate the soft palate and reduce palatopharyngeal incompetency. The patient was advised to use the plate during all hours of the day except during meal times. After a period of adjustment to the palatal plate of about 15 days the patient underwent a clinical and instrumental re-evaluation.

Results: Qualitative analysis by the patient and by the specialists has found an improvement of intelligibility of speech and a reduction of hypernasality. These findings were supported by objective evaluations carried out with

rhinomanometry and acoustic analysis of the voice. In particular, the rhinomanometry showed a marked reduction of nasal airflow and increased nasal resistance. Acoustic analysis of the voice-identified reduction of the parameters relating to voice turbulence index; the spectrographic analysis showed a reduction of background noise and the more obvious differences between minimal pairs.

Discussion and conclusion: We have shown that in selected cases of ALS patients with mild dysarthria associated with hypernasality, the speech intelligibility can be improved by the use of specific palatal prosthesis, greatly raising the quality of life of the patient.

P38 ONE-HANDED FEEDING TUBE ASSIST DEVICE FOR ALS CLIENTS WITH DECREASED HAND FUNCTION

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Keywords: tube feeding, nutrition, hand function

Background: Many ALS clients have feeding tubes placed to supplement their nutrition and hydration. In 2012–2013, Vancouver Coastal Health ALS Centre saw 87 clients with feeding tubes representing 22% of the provincial caseload. Prior to tube placement, the ALS Centre Occupational Therapist (OT) assesses clients' hand function to use a syringe and open a feeding tube end. The ALS Centre Dietician (RD) provides tube feeding education and supplies to support clients at home. A client must be able to flush the tube with water to maintain a minimum standard of tube care. Independence is compromised when clients have hand weakness. Not all clients in the province of British Columbia have access to home support workers who are trained to assist with tube feeding due to risk management concerns, and a reluctance for home support agencies to institute a delegation of task (DOT) for tube feeding assistance. An alternative solution was required to support ALS clients with limited hand function to be independent in basic tube feeding activity.

Objective: To develop a device to assist clients with decreased hand function to independently manage their tube feeds at home.

Method: In 2012, Orthotists at G. F. Strong Rehabilitation Centre developed a tube feeding stand to assist a stroke client who only had the use of one hand. The device allowed this client to be independent and he was discharged home rather than requiring facility placement. The device consisted of a board which holds a bowl of water to allow for flushing the tube, a custom bracket to help draw water with the syringe, a stand to hold the feeding tube end, and a mount to stabilize the syringe and plunge water into the feeding tube.

Results: Four ALS clients have since trialled the device with varying degrees of success. Three clients/caregivers had insufficient hand dexterity to operate the system. One client is presently using the device. In May 2013, the ALS Society of British Columbia provided funding to have an ambidextrous device made. The device can now be loaned out to clients for the time period they have adequate hand function to independently administer their tube feedings.

Conclusion: This unique device allows ALS clients to independently optimize their nutrition via their feeding tubes. Future considerations may include developing specific criteria to predict which ALS clients could benefit from using the device, and modifying the system so it can be fixed to a table for seated or standing use.

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THEME 2 RESPIRATORY AND NUTRITIONAL MANAGEMENT

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THEME 2 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P39   INITIAL RESULTS FROM THE HIGH FAT/HIGH CALORIE VERSUS OPTIMAL NUTRITION IN ALS CLINICAL TRIAL

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Keywords: hyperalimentation, metabolism, diet

Background: Unintentional weight loss is a significant clinical problem in amyotrophic lateral sclerosis (ALS). Mild obesity is associated with improved survival in ALS in a dose-dependent manner. Animal studies have shown that diets high in fat calories increase median survival time of $SOD1^{G93A}$ mice. We hypothesized that hyperalimentation might improve survival in ALS.

Objectives: The High Fat/High Calorie Diet versus Optimal Nutrition in ALS clinical trial (NCT00983983, funded by the Muscular Dystrophy Association), is a phase II, double-blind, placebo-controlled randomized clinical trial to study the safety, tolerability and feasibility of hyperalimentation using standard and high-fat diets. Secondary outcome measures include changes in body mass, body composition, respiratory function, disease progression, and survival.

Methods: Patients with ALS receiving percutaneous enteral nutrition were randomized 1:1:1 to 100% of caloric needs using Jevity 1.0 (control diet), 125% of caloric needs using Jevity 1.5 (high calorie diet), or 125% of caloric needs using Oxepa which contains 55% fat calories (high fat/high calorie diet) (all Abbott Laboratories, Abbott Park, IL) and followed for four months. Energy needs were estimated based upon the Measured Resting Energy Expenditure or participants' current nutrition requirements, whichever was greater. Participants and evaluating investigators were blinded to treatment assignment. Primary endpoints included adverse events (AE) and compliance rates.

Results: A total of 28 participants were screened and 24 participants were enrolled between December 2009 and October 2012. Seven participants were randomized to the control diet, nine to the high calorie diet, and eight to the high fat/high calorie arms, although four participants (1, 1, and 2, respectively) withdrew after randomization but before starting study diet. Baseline demographics were similar among the three study arms. Four participants (3, 0, and 1, respectively) discontinued the study diet due to adverse events. Overall, the tolerability rate was 43% vs. 89% vs. 63%. There were four deaths during the study, all due to respiratory failure: 3 in the control, 0 in the high calorie, and 1 in the high fat/high calorie arm. No deaths were considered related to study diet. There were 42 AE and 9 SAE in the control, 24 AE and 0 SAE in the high calorie and 49 AE and 3 SAE in the high fat/high calorie group. The most common AE were gastrointestinal (71% vs. 44% vs. 63% of participants experiencing at least one AE) and most common SAE were pulmonary (43% vs. 0% vs. 25%).

Discussion: This is the first clinical trial conducted in ALS patients who require percutaneous enteral nutrition. The early results of this study demonstrate significant barriers to enrolment and a high frequency of pulmonary and gastrointestinal AE. Results of functional and other secondary outcome measures will also be presented.

P40   EFFECT OF BCAA SUPPLEMENTATION ENRICHED OF LEUCINE IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: leucine, branched chain amino acids (BCAA), body mass index(BMI)

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease with high risk of malnutrition. Malnutrition itself can produce neuromuscular weakness and adversely affect patients' quality of life, thereby creating a vicious circle. The nutritional status of ALS patients (PALS) can be assessed with dietary review and measurements of weight (w) and height (h): a Body Mass Index ($BMI = w/h^2$) below 18.5–20 kg/m^2 indicates a state of malnutrition. Arm muscle area (AMA) and bioelectrical impedance analysis can assess body composition.

Objective: The purpose of this study was to evaluate the efficacy of Branched Chain Amino Acid (BCAA) supplementation on nutritional and functional parameters of PALS.

Method: A prospective randomized double-blind study was performed with 12 PALS, divided into two groups: the treatment group received BCAA supplementation (80 mg/kg bodyweight/day with ratio leucine/isoleucine/valine of 6/1/1), whereas the control group received placebo. They underwent prospective nutritional and functional assessment for 12 months. All measurements were taken before (baseline), every 4 months (mid-points) and at the end of the trial (end-point).

Results: Patients in the treatment group showed weight gain, increased BMI, and increased AMA. In the control group, biochemical parameters did not change. The other blood biomarkers did not show any significant change throughout the trial.

Discussion and conclusion: This pilot study indicates that leucine could be proposed as a promising pharmaconutrient in the prevention and treatment of sarcopenia, and the BCAA supplementation may be useful in the nutritional therapy of PALS.

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P41   PROSPECTIVE STUDY OF HYDRATION STATUS IN ALS PATIENTS

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Keywords: hydration, water turnover, survival

Background: Dehydration is of concern in ALS and is frequently overlooked (1). Suboptimal hydration predisposes to enhanced morbidity and possibly mortality. The Center for Medicare and Medicaid Services (CMS) has benchmarks for assessing dehydration in elderly.

Objectives: Compare water turnover and total body water (TBW) in ALS patients and healthy controls using labeled water (LW) (2,3); Identify at-risk patients for dehydration Validate water intake predicted from published CMS equations (4,5) against LW.

Methods: Five centers participated in study in definite or probable ALS. Parameters were examined at baseline and every 4 months for 12 months. Paired t-test and Pearson correlation analyzed TBW in patients and controls; t-test compared risk of abnormal water turnover from known prognostic variables in ALS. Absolute and percentage differences in water intake between LW and predictive CMS equations were compared using paired t-test. Significance at $p < 0.05$ was accepted.

Results: Fifty-seven patients revealed at baseline: males 60%; bulbar-onset 33.3%; mean ($\pm$ SD) age 59.5 ± 12.0 years; disease duration 24.3 ± 16.2 months; BMI 27.3 ± 4.7 kg/m; ALSFRS-R 35.2 ± 5.7; and FVC $75.2 \pm 16.2\%$. Patients showed significantly different values, as follows (normal controls vs. ALS): BMI 25.7 ± 3.8 vs. 27.5 ± 4.6; fat-free mass 49.6 ± 12.3 vs. 47.2 ± 10.9 (kg); TBW 36.3 ± 8.9 vs. 34.4 ± 7.9 L; and water turnover 3.04 ± 0.66 vs. 2.75 ± 0.85 L. Water turnover was significantly lower in females, bulbar-onset patients, and in those with lower BMI and FVC, the predicted intake of water ranged from -12.9% to $+36.3\%$. Data on longitudinal changes in hydration status and effects on disease progression and survival will be presented.

Discussion and conclusion: By LW, water turnover and TBW are 10% and 6% lower in ALS patients than in matched controls. About 20% of patients show decreased water turnover. Females, bulbar-onset patients, and those with lower BMI, ALSFRS and FVC are at-risk for dehydration.

Published equations in normal controls inaccurately predict water requirement in ALS patients.

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P42   PERFORMANCE OF NUTRITION TEAM SPECIALIZED IN TREATMENT OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS-ALS

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Keywords: enteral nutrition, body mass index, nutritional status

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by a loss of motor neurons in the cerebral cortex, brainstem and spinal cord, leading to skeletal muscle atrophy, paralysis and death. The clinical onset can be bulbar, affecting swallowing and phonation, or spinal, presenting as skeletal muscle atrophy. Dysphagia is a condition that leads to decreased food intake and body mass, malnutrition and dehydration. Weight and muscle loss are related to the disease progression. For this reason, some therapeutic measures are adopted in order to minimize this impact as the systematic nutritional monitoring.

Objective: To present clinical and nutritional conditions of the patients accompanied by experts in nutrition in ALS.

Methods: There were 28 patients evaluated: 23 (82%) had appendicular involvement, 10 (43.5%) women and 13 (56.5%) men; 5 (18.0%) had bulbar involvement, three (60%) women and two (40%) men. The nutrition evaluation included: anamnesis, assessing the appetite and food consistency, meal frequency, dysphagia, nutritional status, intestinal function and functionality scale (ALSFRS-R). The assessments were conducted by a dietitian team of the Neuromuscular Disease Research Department of University Federal of São Paulo.

Results: Co-morbidity was observed in 10 (36%) patients; seven (70%) had hypertension and three (30%) diabetes mellitus. The average duration of the disease was 68 months (6–264). The appendicular involvement, 14 patients (50%) with initial commitment of upper limbs, nine (32%) with lower commitment and five (18%) bulbar involvement. Dysphagia was observed in 83% of patients. As NIV, 14 (50%) of patients hadn't indicated. As appetite, reduction was observed in 43%, increased in 14% and no changes in 43% of patients.

Consistency modification diet was found in 12 (56%) patients, to pasty or liquid diet. The exclusion of oral diet was found in four (14%) patients. Only five (18%) patients had the proper diet, fractionation with 3 hours range. Constipation was present in the majority (75%). An alternative way of feeding was found in 43% of cases, with the majority (72%) with GEP. The average BMI was 22.7 Kg/m^2 (16–29) and the average ALSFRS score of 15 (10–42).

Discussion: The change of nutritional status in ALS is a multifactorial cause. Factors such as presence of co-morbidities, eating habits (mainly the choice of food and nutritional status) and prior to onset of disease. The form of manifestation of the disease and the intensity of the symptoms are also factors that may influence on the nutritional development.

Conclusions: Nutritional monitoring carried out by expert team can ensure the monitoring of factors that contribute negatively to nutritional status and that can accelerate the evolution of disease.

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P43   GASTROSTOMY USE IN MND: FACTORS INFLUENCING DECISIONS, CHALLENGES AND BENEFITS FROM THE PERSPECTIVE OF PATIENTS AND THEIR INFORMAL CARERS

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Keywords: gastrostomy, timing, impact

Background: Gastrostomy feeding is commonly used to support MND patients with severe dysphagia. The effect of gastrostomy on quality of life for patients with MND is uncertain. Furthermore, there is a paucity of MND literature describing patient and carer experiences in relation to decision-making processes about the timing of gastrostomy insertion; as well as self-perceived benefits and challenges following gastrostomy.

Objectives: To explore the experience of patients with MND, and their informal carers, regarding gastrostomy insertion including the factors influencing gastrostomy timing and the impact of gastrostomy feeding on everyday life.

Methods: Qualitative, in-depth, semi-structured interviews with patients, and carers, were carried out to explore and elicit the perceptions of patients, and their carers in relation to gastrostomy timing and the impact of gastrostomy on their lives.

Results: Ten patients and eight informal carers took part in the study. Factors such as prolonged meals, the task of food preparation, chocking/aspiration and weight loss acted as triggers for undergoing a gastrostomy. Reluctance to give up oral feeding, not realising the potential benefits and negative perceptions of gastrostomy influenced a decision to delay the procedure. Initial gastrostomy education at hospital was limited, non-structured and opportunistic. Home-based education was more comprehensive but on occasions difficult depending on individual patient needs and the geographical area in which participants lived. In addition, clinical complications,

practical issues, time restrictions imposed by strict feeding regimes and the loss of the ability to share a meal, detrimentally influenced psychosocial quality of life. However, the establishment of a safe alternative route for feeding and medication and the reduced worry over difficult meals and weight loss positively influenced quality of life.

Discussion: From a clinical perspective, offering early gastrostomy insertion to patients presents a number of potential benefits. However, this advice does not outweigh the personal perceptions and psychosocial factors for patients. It is important that clinicians are aware that a broad range of factors may act as barriers to timing-related decision-making and that due to the nature of the disease patients may require a greater amount of direction from health professionals. Once a decision to undergo gastrostomy has been made, information provision prior to insertion is important in reassuring patients and reducing their anxiety. In addition, it is essential that patients and carers are adequately trained before being sent back home regardless of the help support that follows up in the community. Continuous support is the key in the transition from oral to gastrostomy feeding.

Conclusions: Understanding the factors which influence decision-making as well as the challenges and benefits of gastrostomy for patients and their carers is important for information and care provision by health care professionals.

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P44   ANALYSIS OF PREDICTIVE FACTORS FOR SURVIVAL PROGNOSIS AT THE TIME OF PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: percutaneous endoscopic gastrostomy, survival prognosis, arterial carbon dioxide pressure

Background: Malnutrition is an independent prognostic factor for survival in patients with amyotrophic lateral sclerosis (ALS), and percutaneous endoscopic gastrostomy (PEG) in an early stage has been recommended. However, evidence for optimal timing of PEG has not been established.

Objective: To analyze predictive factors for survival periods after the time of PEG and to search optimal timing of PEG in patients with ALS.

Methods: In total, 105 consecutive patients with sporadic ALS who underwent PEG in our hospital were analyzed (47 men and 58 women; mean age at PEG 70 years, range: 32–90 years). We analyzed effects of clinical parameters on survival periods after PEG to endpoints for follow-up, using Kaplan–Meier survival analysis method and log-rank test. The endpoints were defined as death or ventilator use. The analyzed factors were sex; age at onset (≥ 65 or < 65 years); onset region (bulbar or spinal); forced vital capacity (% FVC); arterial carbon dioxide pressure (PCO_2); body mass index (BMI); and ALS-functional rating scale (ALSFRS) at the time of PEG. Thereafter, univariate and multivariate analyses for

survival periods were performed using the Cox proportional hazard model.

Results: The log-rank test showed significant effects of PCO_2 (> 40 mmHg, p = 0.0052), %FVC (< 38%, p = 0.0018), onset region (spinal onset, p = 0.0135) on the survival periods after the time of PEG. The bulbar-onset group showed a better prognosis after the time of PEG than the spinal-onset group, although the time of PEG was earlier in the bulbar onset than the spinal onset. The analyses of correlation between clinical parameters and survival periods after PEG showed a significant correlation for PCO_2 (Spearman's rank correlation test, p = 0.0039) and non-significant weak correlation for %FVC (p = 0.0563) and BMI (p = 0.0665). Multivariate analysis by the Cox proportional hazard model, when including the parameters of sex, onset age, onset region, BMI (≥ 18.5 or < 18.5) and PCO_2 (< 40 mmHg or ≥ 40 mmHg), showed statistically significant effects of the onset region (spinal onset) and PCO_2 (≥ 40 mmHg) on survival length after PEG.

Conclusions: PEG is recommended at an early stage of ALS at least before arterial carbon dioxide begins to accumulate. Bulbar onset may predict better survival prognosis after the time of PEG than spinal onset, which may indicate that PEG should be introduced earlier in spinal onset patients than bulbar onset ones when PCO_2 begins to be elevated from normal stable levels for individual patients. BMI itself may not predict post-PEG survival prognosis. Constant evaluation of PCO_2 is recommended as well as %FVC and BMI, particularly at stages of malnutrition and respiratory deterioration.

P45   DAILY ENERGY EXPENDITURE ASSESSED BY DOUBLY LABELED WATER METHOD IN JAPANESE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: malnutrition, total energy expenditure, doubly-labeled water

Background: Malnutrition is an independent prognostic factor for survival in patients with amyotrophic lateral sclerosis (ALS), and disease-specific hypermetabolism has been thought to underlie the malnutrition. To prevent progressive weight loss, percutaneous endoscopic gastrostomy (PEG) and nutritional intervention at early stage is recommended. However, precise amount of daily energy intake has not been established particularly in Asian patients with ALS.

Objective: To clarify the amount of daily energy expenditure in Japanese patients with ALS in various stages.

Methods: Seventeen patients with sporadic ALS were enrolled in the study (four men and 13 women, mean age: 67 years, range 46–78 years). Three patients were receiving

non-invasive positive pressure ventilation (NPPV). Six patients had PEG at the time of examination, and 5 of 6 were receiving only enteral feeding through PEG. No patients underwent tracheostomy. Total energy expenditure (TEE) was measured using the doubly labeled water (DLW) method. Patients were given a dose of 0.14 g of ^{18}O and 0.06 g of ^2H per kg body weight orally or through PEG catheter. Urine was collected before and after administration of DLW for 14 days (a total of eight samples). We measured isotope ratio of urine ^{18}O and ^2H, and the carbon dioxide production rate was calculated. TEE (kcal/day) was calculated using modified Weir's formula.

Results: The mean body mass index (BMI) was 19.4 kg/cm^2 (range: 14.0–24.3), and the mean free fat mass (FFM) was 31.1 kg (range 20.1–46.8). The mean TEE was 1532.7 kcal/day (range: 897.9–2195.1), and the mean TEE per body weight (TEE/BW) was 32.9 kcal/kg/day (range: 25.7–41.5). There was a significant correlation between BMI and TEE (Spearman's rank correlation test, p < 0.05). The activity index for the patients was 1.1–1.3. TEE/BW in the patients was higher than standard values of TEE/BW in healthy Japanese subjects when the activity index was estimated as 1.2. (Standard resting energy expenditure in Japanese healthy subjects older than 50 years was reported as 21.5 kcal/kg/day in man and 20.7 in women.) TEE/BW in the patients was not significantly correlated with ALS functional rating scale and disease duration. In addition, TEE/BW was higher than calculated TEE/BW using Harris-Benedict formula.

Conclusions: Japanese ALS patients also showed increased energy consumption regardless the stages of disease. Before tracheostomy, ALS patients show constantly high energy expenditure, which might be attributed to multiple factors: disease specific hypermetabolism, increased respiratory muscle effort, and elevated sympathetic tone. Exact daily necessary energy amount should be established in the future in each stage and in each nutritional or respiratory state.

P46   EFFECTS OF COUGH AUGMENTATION ON PULMONARY MORBIDITY, SURVIVAL AND QUALITY OF LIFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN RESPIRATORY FAILURE: A RANDOMISED CONTROLLED TRIAL

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Keywords: cough augmentation, mechanical in-exsufflator, breath-stacking

Background: A major problem faced by patients with amyotrophic lateral sclerosis (ALS) is inability to cough effectively and remove secretions from the airways. In the absence of robust evidence, there is no clear consensus about how best to help these patients. In this randomised trial, we evaluated two cough augmentation techniques, that is, mechanical insufflator/exsufflator (MI-E) versus manual insufflator (breath-stacking technique).

Objectives: To assess the effect of cough augmentation on pulmonary morbidity, quality of life and survival in amyotrophic lateral sclerosis.

Methods: A total of 40 eligible patients were randomised to breath-stacking technique (n = 21) or MI-E (n = 19) and followed-up at three monthly intervals for at least 12 months or until death. All patients were diagnosed with respiratory failure and offered non-invasive ventilation (NIV). The primary outcome measure was number of days with symptoms of chest infection, treated with antibiotics, in the community or in the hospital. Survival and quality of life benefit, assessed by short form 36 mental component summary (MCS) and sleep apnoea quality of life index symptoms domain (sym), are the secondary outcome measures. For survival analysis, data of 20 eligible patients (matched for prognostic variables), offered NIV from the end of recruitment to the interventional arms, were also included.

Results: There were 13 episodes of chest infection in breath-stacking group and 19 episodes in MI-E group (p = 0.87), requiring 90 and 95 days of antibiotics, respectively (p = 0.85). There were six episodes of hospitalizations in each group (p = 0.87). Median survival in breath-stacking group was 535 days, 266 days in MI-E group and 244 days in the control group (p = 0.54). MCS was maintained above 75% of baseline for a median of 329 days in breath-stacking group and 205 days in MI-E group (p = 0.41).

Discussion and conclusion: In ALS patients with respiratory failure, cough augmentation improves survival and maintains quality of life. The survival benefit is superior to the benefit offered by NIV alone. Although episodes of chest infections were fewer in breath-stacking group, duration of antibiotics use and chance of hospitalization in the event of a chest infection was less in MI-E group. The study, although limited in terms of sample size, offers insightful information and supports routine domiciliary use of breath-stacking in patients with ALS requiring respiratory support. MI-E may be useful in a hospital setting, reducing the course of antibiotics and length of stay, in the event of a chest infection.

P47   THE RELATION BETWEEN THE PEAK COUGH FLOW AND THE FUNCTIONAL ORAL INTAKE SCALE ASSOCIATED WITH THE FUNCTIONALITY IN PATIENTS DIAGNOSED WITH MOTOR NEURONE DISEASES

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Keywords: deglutition disorders, cough, deglutition

Background: Dysphagia is a common symptom of Motor Neurone Diseases (MND). There are two important factors that add to the occurrence of difficulty in swallowing: the weakness of oropharyngeal muscle and changes in the respiratory mechanics. The cough is a very important defensive mechanism used for airway protection which can minimize respiratory problems that are secondary to the difficulty in swallowing.

Objective: To analyze the relation between the peak cough flow and the functional oral intake scale associated with its functionality in patients diagnosed with MND.

Methods: There were 37 patients evaluated, 24 (64.8%) had amyotrophic lateral sclerosis (ALS) – 15 (62.5%) women and nine (37.5%) men – and 13 (35.2%) had progressive bulbar paralysis (PBP) – five (38.4%) women and eight (61.6%) men. The swallowing evaluation included anamnesis, with further examination of feeding adjustments made by patients: oral and pharyngeal phase of swallowing; functional oral intake scale (FOIS); peak cough flow (PCF); functionality scale (ALSFRS-R). The assessments were conducted by a swallowing therapist team of the Neuromuscular Disease Research Department of University Federal of São Paulo.

Results: Changes in oral phase happened in 16 ALS patients (66.67%) and 13 PBP patients (100%); changes during pharyngeal phase in 16 ALS patients (66.67%) and 13 PBP patients (100%). Every patient that showed some change in swallowing, some in oral phase and others in pharyngeal phase had to make some adjustment in food consistency in their daily meals. Patients that used an alternative way of feeding presented numbers of PCF below 115 litres/minute (l/min), while patients that used different types of food consistencies (Level 5–7 of FOIS scale) showed PCF above 230 l/min in ALS and PBP. Patients with ALS presented PCF below 100 l/min scored 1.4; 3.4; 6.2 and 11 in matters related to bulbar signs, appendicular and respiratory and the total functionality scale, respectively; from 100 to 200 l/min, 6.82; 7.09; 8.18 and 22.09; above 200 l/min, 9.75; 12.86; 11.14 and 33.71. PPB patients that presented PCF below 100 l/min scored 3; 13.3; 7.3 and 23.67 in matters related to bulbar signs, appendicular and respiratory and the total functionality scale, respectively; from 100 to 200 l/min, 4; 16.5; 9.5 and 30; above 200 l/min, 7.2; 16.2; 10.4 and 33.8.

Discussion: Changes in the oral and pharyngeal phase of swallowing can compromise the patient's nutrition and hydration, by reducing oral intake and they can also compromise the respiratory function, because of the bronchoaspiration. Feeding adjustments combined with an efficient cough can minimize complications secondary to dysphagia.

Conclusions: There was a match between the adjustments in the food consistencies with the swallowing difficulties and with the PCF measures associated with the functionality scale (ALSFRS-R) both general and specific.

P48   THE BALLOON – BASED MANOMETRY: EVALUATION OF SWALLOWING IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: swallowing, balloon-based manometry

Background: Patients with bulbar onset present firstly with speech disorders, swallowing problems and dysphonia. The swallowing problems in ALS patients are frequently estimated using manometric parameters.

Objective: The aim of the study was to analyse the disturbances of the oro-pharyngeal swallowing phase of dysphagia in ALS patients by the using balloon-based manometry.

Material and methods: Thirty-six ALS patients were diagnosed in the Department of Neurology. There were 21 male (58.3%) and 15 female (41.7%) patients, aged between 26 and 78 (mean: 59.9 ± 11.4 years). Complete manometric examinations were performed thrice: at the start point of the research, and then 6 and 12 months later.

Manometric examinations of oral and pharyngeal part of gastrointestinal tract were carried out by using the esophageal ballon-based method with four balloon transducers located 5 cm away from each other, according to the procedure described in the literature. The transducers measured the changes of the pressure on the base of the tongue, the hypopharynx and the entrance to the larynx, the upper esophageal sphincter (UES) and the esophageal body. The following manometric parameters were analysed: the maximal contractions of the base of tongue (CBT) and the UES resting preasure (RP); hypopharyngeal suction pump (HSP); oropharyngeal, pharyngeal and hypopharyngeal transit time (OTT, PTT, and HTT, respectively); and velocity for bolus. The manometric examination was carried out with the use of Polygraf ID. The results were recorded in a database installed in a computer working on Polygram 98/Polygram Net.

Results: Significant weakness of CBT ($p = 0.007$), decrease of HSP ($p = 0.006$) and decrease of velocity of bolus transit ($p = 0.001$) were particularly marked between the first and the third examination.

Discussion: On the basis of the manometric examinations, disturbances of contraction of the base of the tongue, hypopharyngeal suction pump and pharyngeal transit time were shown in bulbar onset ALS patients. The results obtained from manometric examinations confirm progression of swallowing disorders.

Conclusion: The balloon-based manometry examination is useful in the estimation of disturbances with swallowing in ALS patients.

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P49 COORDINATION OF SWALLOWING AND BREATHING IN PATIENT WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: dysphagia, swallowing, breathing

Background: The coordination of swallowing and breathing is essential to the prevention of pulmonary aspiration. Among the diseases that can cause an alteration of this fine coordination mechanism there are numerous neurological diseases, including amyotrophic lateral sclerosis (ALS).

Objectives: The aim of the study is to analyze the coordination of swallowing and breathing in ALS patients.

Methods: Combined Fiberoptic Endoscopic Evaluation of Swallowing, respiratory phase and submental surface EMG recordings were analyzed in 13 patients with ALS. The following swallow tasks were considered: spontaneous swallow during a 5 minutes recording with and without endoscopic control and five voluntary dry swallows; single as well as sequential deglutition with thin liquid (5, 10, and 50 ml) and semisolid bolus (5 and 10 ml). For each swallow, offline from the digitized recordings, the following variables were measured: (1) duration of the sustained swallowing apnea; (2) numbers of swallows/bolus; (3) respiratory phases surrounding each swallow. Patients were divided into those who had a "safe" swallow and those with an "unsafe" swallow, according to the grade obtained in the Penetration Aspiration Scale (PAS) and in the Dysphagia Outcome and Severity Scale (DOSS). Respiratory parameters were collected. Mann–Whitney and Spearman nonparametric tests were used for statistical analysis.

Discussion and results: No difference was found in apnea duration and number of swallows measured with endoscopic control and those measured without it ($p = 0.281$). A total of 644 swallows with either thin liquid or semisolid were analyzed. In 50.1% of the patients, the deglutition was followed by the expiration phase. Percentage of inspiration-deglutition-inspiration pattern depended on viscosity and decreased with semisolid bolus. The average of swallowing apnea duration was 1.7 seconds. This time lapse depended on swallowing task, bolus viscosity and size. The average ratio of swallow/bolus was 2.06. This ratio doubled in patients with an "unsafe" swallow and depended on bolus viscosity and size. A strong correlation between SpO2 and swallowing apnea duration on Spearman test was found ($r = 0.8$). No correlation between swallowing apnea duration and swallows/bolus ratio was found.

Conclusions: Many parameters describing coordination of swallowing and breathing in patient with ALS are modified compared with the same data found in the literature on healthy adults. Therefore the planning of speech and language treatment should include strategies to improve the coordination between swallowing and breathing in order to reduce the risk of pulmonary aspiration.

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P50   CORRELATION AMONG FORCED VITAL CAPACITY, RESTING ENERGY EXPENDITURE AND SEGMENTAL TRUNK BIOELECTRICAL IMPEDANCE ANALYSIS IN ALS PATIENTS FOR PREDICTING CLINICAL DISEASE PROGRESSION: A PRELIMINARY STUDY

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Background: The typical ALS disease course is characterized by progressive irreversible muscle wasting of the limbs, torso, abdomen, and oropharyngeal muscle regions, and often a decline in nutritional status that is inadequately addressed in clinical practice, even though it is a significant and independent prognostic factor for survival.

Objective: To clarify if Resting Energy Expenditure (REE), Forced Vital Capacity (FVC) and segmental trunk Bioelectrical Impedance Analysis (stBIA) are correlated with clinical disease progression in ALS patients (PALS).

Methods: In 12 patients (mean age $\pm$ SD: 52.1 ± 11.5 yrs; 7 males and 5 females) with definite ALS, neurologic deficit was quantified by manual muscular testing of all extremities and the neck, as defined by the Medical Research Council. All patients were stable in pharmacological medication (50 mg riluzole twice a day); no patient received any steroid drug treatment. FVC was measured with a pneumotachograph system (Medical Graphics, St Paul): findings were expressed in relation to a theoretical calculated index value. Indirect calorimetry was performed with a VO2000 (Medical Graphics, St Paul) that was calibrated each morning before the measurements were made (1): measurements were accepted if the results were at a stable plateau for 20 min or more. The measured REE (mREE) was compared with REE obtained from a control population volunteers and with REE calculated (cREE) by using the Harris–Benedict equations. In stBIA (50 kHz) the four source electrodes and the combination of eight detecting electrodes used in this study allowed separation of the trunk into five parts, the Z of each part could then be determined (2). All PALS were tested every 4 months: in such a preliminary work a period of 24 months has been analyzed.

Results: mREE/cREE and mREE data appeared to remain stable. FVC was not associated with mREE, but with stBIA: in fact, both FVC and stBIA significantly decreased according to the PALS clinical worsening.

Conclusion: Data analysis suggests a significant ($p < 0.01$) correlation (0.96) among FVC, stBIA and clinical disease progression.

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P51   RESPIRATORY IMPAIRMENT IN PATIENTS WITH MOTOR NEURON DISEASE: REFERRAL PROCESS AND FIRST ASSESSMENT AT A HOME VENTILATION SERVICE IN THE NETHERLANDS

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Background: In order to further improve the care for Dutch patients with motor neuron disease, we assessed the first evaluation at a large ventilation clinic in the Netherlands.

Objective: (i) To determine what proportion of patients with motor neuron disease would benefit from earlier referral and (ii) to examine the patient preferences regarding ventilatory support.

Methods: Observational study at a single centre with a catchment area of 7.6 million inhabitants. Data on disease status, the referral process and patients' preferences regarding ventilatory support were collected during the first home ventilation service (HVS) assessment and analysed for correlation with the presence of daytime hypercapnia and suspected nocturnal hypoventilation. The latter conditions require immediate (within 48 h) or subacute (within 3 weeks) initiation of ventilatory support.

Results: Vital capacity (in percentage of predicted value, VC % pred) was assessed by referring physicians in 84% of the 217 referred patients; the mean VC % pred was 69% (SD 16). One-hundred and ninety-one patients attended the first HVS assessment without ventilatory support, at a median of 21 days following referral: 18% had respiratory failure (daytime hypercapnia); 19% had normocapnia but were suspected of nocturnal hypoventilation; 63% had normocapnia without symptoms. Following the HVS assessment, 25 patients (13%) declined home mechanical ventilation; this occurred more often in patients with (14/70) compared with patients without respiratory impairment (11/121; $p < 0.05$).

Discussion and conclusion: A meaningful proportion of patients who desire ventilatory support are referred to a ventilation clinic after already developing respiratory failure. Future studies could examine means, including more sensitive respiratory measures, to detect those patients who could benefit from earlier referral.

P52   RESPIRATORY ASSISTIVE CARE APPLIED TO HYPOVENTILATION WITHOUT SIGNIFICANT DIAPHRAGMATIC DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: respiratory impairment, phrenic nerve motor response, respiratory function tests

Background: It has been reported that diaphragm compound muscle action potential (DCMAP) could be a potential indicator of respiratory impairment in ALS. However, we have experienced some ALS patients without correlation between DCMAPs and the other biomarkers such as % forced expiratory vital capacity (%FVC) and sniff nasal-inspiratory pressure (SNIP) in accordance with the respiratory dysfunction.

Objectives: We performed a longitudinal study to elucidate correlations between respiratory impairment and respiratory biomarkers including DCMAPs at the initiation of respiratory assistive care in ALS.

Methods: We enrolled 42 ALS patients from October 2006 to December 2011, and performed the follow-up study until March 2013 except for the death. All patients were assessed at least thrice approximately every 6 months. Additional assessments were performed when the respiratory assistive care was given to the patient with respiratory insufficiency according to the guidelines. Each assessment included full neurological examinations, application of the ALSFRS-R, phrenic nerve conduction study, respiratory function tests and nocturnal pulsed oximetry. The statistical analysis was performed using the Mann–Whitney U-test, Wilcoxon matched-pairs signed-ranks test and chi-squared test.

Results: The patients were divided into three groups according to the timing of respiratory assistive care such as non-invasive ventilation (NIV) or tracheostomy. Eleven patients initiated the respiratory care while DCMAP remained above 0.2 mV in group A. Eleven patients initiated it when DCMAP decreased below 0.2 mV in group B. Twenty patients received no respiratory care in the observation periods. There was no significant change of the onset age, the disease duration, ALS-FRS-R, %FVC and SNIP between group A and B. Group A had a higher incidence of bulbar signs and positive plantar responses compared with those of group B. The mean DCMAP was significantly higher in group A than in group B at the initiation of respiratory assistive care. The deterioration of respiratory parameters had a linear correlation with the decrease of DCMAPs according to the progression of hypoventilation in group B. However, group A showed no significant change of DCMAP during the observation periods despite the progression of hypoventilation.

Discussion and conclusion: The respiratory impairment with preserved DCMAPs was seen in some ALS patients, especially in the patients with bulbar involvement and/or marked pyramidal signs. We should note the respiratory failure without significant diaphragmatic dysfunction in ALS.

P53   NON-TERMINAL WEANING FROM INVASIVE VENTILATION IN ALS/MND

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Keywords: ventilation, quality of life, weaning

Background: Decisions about use of non-invasive and invasive ventilatory support are among the most challenging for patients and families facing ALS (1). The medical literature on ventilator use in ALS via tracheostomy offers descriptions of its feasibility, including in the home setting, and patterns of withdrawal (2,3). It is generally presumed that invasive ventilator support will be continuous and permanent.

Objectives: To describe successful weaning from invasive ventilation in ALS.

Methods: Chart review of cases diagnosed with ALS who had patient-requested weaning of ventilator use via tracheostomy, not associated with an end-of-life care plan, at one U.S. Veterans Administration ALS program, from April 2012 to March 2013.

Case report: Four cases were identified. Case 1: 64-year old (y.o.) presented with respiratory failure, weaned from continuous to nocturnal ventilation 190 days after tracheostomy. The patient continues to live at home, ambulatory. Case 2: 67 y.o. presented with respiratory failure. Began weaning 4 days after tracheostomy and completely withdrew ventilator over several months and returned home. The patient was later re-diagnosed with Guillain–Barre instead. Case 3: 45 y.o. presented with weakness received tracheostomy 4 years later. One year after having nocturnal ventilation in a nursing facility far from home, he was weaned completely and returned to nursing facility near family. Case 4: 67 y.o. presented with weakness, received tracheostomy 2 years later. The patient was weaned to nocturnal ventilation only 7 days later and lives in a nursing facility.

Discussion: In a variety of circumstances, patients with ALS already on invasive ventilation can be weaned from substantially or completely. This can occur in the days after tracheostomy or even many months later. Without consideration of future weaning, a patient with ALS in respiratory failure may consider death preferable to long-term ventilation. Our experience with two cases presenting with respiratory failure are contrary to prior case series suggesting dire outcomes (4). Quality of life was significantly improved with opportunities to be off mechanical ventilation.

Conclusions: Despite progression of disease in ALS, initiation of invasive mechanical ventilation via a tracheostomy may be later followed by successful weaning, to permit increased mobility, physical comfort and greater options for care settings. Decisions about initiating mechanical ventilation should include the possibility of future partial or total weaning with prolonged survival.

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P54 NON-INVASIVE VENTILATION IN MOTOR NEURONE DISEASE: ONE CENTRE'S EXPERIENCE

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Keywords: NIV, bulbar, survival

Background: Respiratory muscle weakness is a major feature of Motor Neurone Disease (MND) and is a strong predictor of quality of life and survival. Studies have shown that non-invasive ventilation (NIV) may be associated with a gain in survival and improved quality of life (1). NICE guidelines (2) suggest that the ongoing management and treatment for a patient with MND (pwMND) should include provision of NIV for suitable patients. Increasing use of NIV in MND is now being reported (3).

Objectives: To report on our centre's experience of using NIV in a group of pwMND who had developed respiratory failure, assessing the effects of different factors on survival.

Methods: Routine data collected on consecutive pwMND who underwent a trial of NIV therapy for respiratory failure between February 2009 and April 2012 were analysed using SPSS Statistics 19. Survival status was noted on 30 April 2013. The effect of mean overnight pCO2 prior to initiation of NIV, bulbar status and compliance to therapy on survival was assessed using Kaplan–Meier survival curves. Median survival and percentage survival at 1 year was calculated for a group of patients who had been followed up for at least one year.

Results: Trials of NIV were performed for 117 pwMND during this period. One hundred and eight patients successfully completed the trial and were issued with machines for home use. Patients were predominantly male (72M/36F) with a mean $\pm$ sd age of 66 ± 12 years (range: 25–95) and a mean $\pm$ sd overnight pCO2 at initiation of 6.85 ± 0.87 kPa.

Kaplan–Meier survival curves showed that survival was significantly related to therapy compliance. Bulbar status was not a significant factor ($p = 0.112$), but there did appear to be a trend for less survival benefit in those patients with bulbar symptoms. There was no effect shown with pCO2, age or sex. In the group of patients who had been followed up for at least one year ($n = 52$) median survival was 295 days and percentage survival at 1 year was 40.7%.

Conclusions: Survival of this group of patients on NIV compares well with published trials. Qualitative data from a subgroup of this cohort suggest that extended survival is not at the expense of quality of life. In contrast to previous reports, patients with bulbar symptoms at the start of trial benefited from therapy. NIV should be considered for all MND patients with respiratory failure.

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P55 BRAZILIAN NON-INVASIVE VENTILATION ASSISTANCE PROGRAM FOR PATIENTS WITH MOTOR NEURONE DISEASES, IN THE CITY OF SÃO PAULO

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Keywords: NIV, survival, dyspnea

Background: The muscle weakness in Motor Neurone Diseases (MND) may compromise upper and lower limbs, pharyngolaryngeal and respiratory muscles. The changes in respiratory function may negatively affect the quality of life and the survival of MND patients. For this reason, some therapeutic measures are taken, aiming to minimize these effects, which is the case of intermittent positive pressure ventilation.

Objective: To describe the Non-Invasive Ventilation Assistance Program for patients with MND, established by the public health system and it was conducted in São Paulo, Brazil.

Methods: This is a descriptive study that delineated the influx of clinical assessment and ventilatory assistance. A program of ventilatory assistance was created to enable the supply of the bilevel ventilation equipment nationwide to all MND patients. The National Regulation determines that every patient diagnosed with MND is entitled to the bilevel ventilation equipment, in addition to its upkeep and the patient's follow-up at home.

Results: In the Neuromuscular Disease Research Department of University Federal of São Paulo, MND is diagnosed by a neurogical assessment. Afterwards the patient is conducted to a multidisciplinary assessment. After this initial assessment, quarterly follow-up visits are scheduled so that proper guidance can be given, according to the medical condition. The established criteria for the use of non-invasive mechanical ventilation (NIMV) are symptoms of alveolar hypoventilation (tiredness, dyspnea, and morning headache); waking up with dyspnea and tachycardia; sleepiness; frequent pneumonia; inefficient airway clearance. With a value of FVC (Forced Volume Capacity) of approximately 50%, the patient

is conducted to a registered public health care, where the respiratory measures will be registered before the patient can be entitled to a bilevel positive airway pressure with nasal and oral mask and an integrated heated humidifier. The pressure setting is also adequate to the patient's needs. A physical therapist visits monthly and there are follow-ups from time to time in health care, to make the reports about the use of the equipment and also pressure setting, if necessary.

Discussion: The goals of non-invasive ventilation are the pulmonary gas exchange, reducing the respiratory work and the improvement of symptoms. The latest facts about the benefits of NIMV for patients with MND diseases is solid and provides improvement to symptoms like chronic hypoventilation in a short amount of time and improves the quality of life and survival. Moreover, the use of NIMV can contribute to the endoscopic gastrostomy tube placement procedure, indicated to dysphagic patients and/or undernourished patients with more than 30% FVC.

Conclusions: The Non-Invasive Ventilation Assistance Program has shown efficacy in the city of São Paulo/Brazil, meeting the expected goals, showing that it is possible to be introduced, ensuring help and improving the quality of life for patients with MND.

P56   PREOPERATIVE TESTING IN ALS PATIENTS PREDICTS DIAPHRAGMATIC RESPONSE TO DIRECT STIMULATION

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Background: Patients with amyotrophic lateral sclerosis (ALS) ultimately develop diaphragmatic weakness and chronic hypoventilation significantly influencing the progression of the disease, and quality of life. Diaphragmatic pacing (DP) has recently been introduced as a way of improving respiratory function. It is unclear which patients are the best candidates for DP.

Method: We performed a retrospective review of pre-operative testing with phrenic nerve conduction study (PNC), and diaphragm fluoroscopy with sniff test (DF) and compared these to intraoperative direct nerve stimulation (DNS) using the NeuRx Diaphragm Pacer System™. PNC responses of greater than 0.2 mV, DF greater than 2 cm excursion and DNS greater than 5 mV/sec were considered to be strong responses. All patients met accepted criteria for device placement based on pulmonary function testing.

Results: A total of 57 hemi-diaphragms in 29 patients underwent PNC and DNS. In 42/57 (73.5%) the PNC and DNS responses were concordant where 17/57 (29.8%) had poor responses in both and 25/57 (43.7%) had good responses in both. In 15/57 (26.3%) the responses were discordant where 10/57 (17.5%) had poor PNC and good DNS, and 5/57 (8.7%) had good PNC but poor DNS. A total of 55 hemi-diaphragms in 28 patients underwent DF and DNS. In 32/55 (58.1%) the responses were concordant where 8/55 (14.5%)

had poor responses in both and 24/55 (43.6%) had good responses in both. 23/55 (41.8%) were discordant where 12/55 (21.8%) had poor DF but strong DNS responses, and 11/55 (20%) had good DF responses but poor DNS. The discordant PNC and DF were not consistent suggesting that the two pre-operative studies were independent predictors of DNS. Of the 38 discordant responses, only 16 were discordant with both tests. Twenty-two were correctly predicted by the other pre-operative test.

Conclusion: Both PNC and DF were predictive of intraoperative response in the majority of cases, with PNC having a stronger correlation. However, both pre-operative studies had false positive and negative results and many patients with poor PNC prediction had good DF prediction and vice versa. This suggests that both studies are needed to determine the suitability of diaphragmatic pacing. We are reviewing the data to determine whether PNC and DF correlate with vital capacity and, inspiratory and expiratory pressures.

P57   NOCTURNAL PULSE OXIMETRY AS AN INDICATOR OF SURVIVAL IN AN ALS CLINICAL POPULATION

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Background: Nocturnal pulse oximetry (NPO) is a simple exam that allows detection of nocturnal respiratory dysfunction in patients with amyotrophic lateral sclerosis (ALS) and has been used to guide the initiation of noninvasive ventilation. However, only few studies specifically investigated the relationship between NPO results and survival.

Objectives: The aim of this study was to evaluate NPO as a useful test to predict survival in an ALS clinical population.

Methods: We prospectively studied 106 patients with ALS. We recorded clinical and demographic data, forced vital capacity (FVC), and survival time. All subjects were submitted to NPO.

Results: We found that patients who demonstrated evidence of SaO_2 desaturation less than 90% for at least one cumulative minute throughout the duration of the nocturnal study survived significantly less than patients with normal NPO results (median survival: 15 months vs. 22 months, respectively; $p = 0.001$). Similar findings were also obtained when patients were divided according to mean overnight $SaO_2 < 93\%$ (median survival: 15 months vs. 20 months, respectively; $p = 0.034$). Interestingly, no differences were found in clinical and demographic variables between the groups of patients, including FVC. Moreover, an altered NPO result (as defined above) was identified as a significant predictor of survival in the multivariate Cox regression model, together with FVC and disease duration.

Discussion: These results confirm the importance of NPO on survival in patients with ALS and may have several consequences for the clinical management of symptomatic care in ALS.

P58   REM SLEEP BEHAVIOR DISORDER AND PERIODIC LEG MOVEMENTS IN SLEEP IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: In the last few years, it has been increasingly recognized that patients with amyotrophic lateral sclerosis (ALS) frequently suffer of sleep-related complaints, including insomnia, restless legs syndrome, and daytime sleepiness, although, in the past, many of the nocturnal symptoms have been mainly ascribed to chronic respiratory insufficiency and hypoventilation. In addition, excluding a few single cases reported, polysomnographic studies did not look for abnormal motor activity during sleep that could be indicative of Rapid Eye Movements (REM) sleep behaviour disorder (RBD) and REM sleep without atonia (RSWA), which are highly prevalent in other neurodegenerative disorders with brainstem involvement. Finally, data on the presence of periodic leg movements in sleep (PLMS) in patients with ALS are sparse.

Objective: We sought to assess sleep characteristics and the occurrence of abnormal muscle activity during sleep such as RSWA, RBD, and PLMS in patients with ALS.

Methods: Forty-one patients with ALS and 26 healthy subjects were submitted to clinical interview and overnight video-polysomnography.

Results: Twenty-two patients with ALS (53.6%) reported poor sleep quality. Polysomnographic studies showed that patients with ALS had reduced total time of sleep, increased wakefulness after sleep onset, shortened REM and slow wave sleep, and decreased sleep efficiency compared to control subjects. Polysomnographic abnormalities were not different in patients reporting good or poor sleep, and were not correlated to clinical and demographic variables. PLMS index was significantly higher in patients with ALS than in healthy subjects, and 22 patients (53.6%) showed a PLMS index greater than 15 vs 4 (15.4%) control subjects ($p < 0.001$). Finally, two patients with ALS (4.9%) had RBD, and two more patients presented RSWA (4.9%), whereas no control subjects showed abnormalities of REM sleep.

Conclusion: Patients with ALS frequently present abnormalities of sleep that can be documented both at the clinical interview and at the polysomnographic evaluation, including insomnia, fragmented sleep, and increased PLMS. Moreover, abnormalities of REM sleep can sometimes be found in these patients.

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THEME 3 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

THEME 3 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P59 FREQUENCY OF COGNITIVE IMPAIRMENT IN ALS PATIENTS

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Objective: To determine the frequency of cognitive impairment in ALS patients.

Methods: We selected 40 patients with the diagnosis of ALS using the "El Escorial" criteria who came for a first appointment to the ALS Unit at Bellvitge University Hospital. They were evaluated both by this Unit and the Dementia Unit. A structured interview, a thorough general and neurological examination, a neuropsychological study, an analysis, a functional and structural neuroradiological study (MRI and PET-18FDG), and an electromyography were all performed. They were evaluated with ALS-CBS test too. We classified the patients according to their cognitive state in three categories: (a) Normal patients, (b) Mild cognitive impairment patients according to consensual criteria by the FTD in ALS workshop in London (Canada), and (c) FrontoTemporal Dementia patients according to Neary's criteria.

Results: The percentages for each diagnosis were as follows: ALS-bi (6.3%), ALS-ci (12.5%), ALS-cibi (43.8%), and ALS-FTD (3.1%). 34.4% of patients were cognitively normal. All the patients with ALS from bulbar onset suffer cognitive impairment. All the patients except those suffering from ALS-FTD had a normal PET-18FDG.

Conclusions: (1) Cognitive impairment is frequent at the moment of diagnosing ALS; (2) It is recommendable to include the cognitive evaluation in the initial approach to ALS patients; (3) The bulbar onset of ALS is more frequently associated with cognitive impairment; (4) PET-18FDG is probably useful in diagnosing ALS-FTD, but not ALS with mild cognitive impairment.

P60 THE COGNITIVE PROFILE OF ALS. UPDATE OF A META-ANALYSIS AS A BASIS FOR A COGNITIVE SCREEN

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Background: In 2008, we performed a literature meta-analysis on the cognitive profile of amyotrophic lateral sclerosis (ALS) which showed significant effect sizes for fluency, executive function, language and memory. For some cognitive domains no firm conclusions could be reached. Since many neuropsychological studies have been published after 2008, we updated the meta-analysis. The results will be used to select tests for an ALS-specific cognitive screen.

Objectives: To update a literature meta-analysis on the cognitive profile of ALS, which is used as a basis for a cognitive screen.

Methods: Pubmed and PsycInfo were searched for articles published since 2008, in English, French, Dutch and German. Articles were selected if healthy volunteers (controls) and non-demented ALS patients, fulfilling El Escorial criteria, underwent at least one validated cognitive test. All tests were categorized in cognitive domains. Effect sizes, expressed as Hedges' g, were calculated per domain. Demographic and clinical data were extracted from the articles.

Results: Twenty-seven new articles were included, resulting in a total of 42 articles (n = 1251 patients and 1111 controls). The median number of neuropsychological (sub) tests administered to patients and controls was 8 (1–34). Patients (63% men, 33% bulbar onset) had a mean age of 58.6 years, a mean educational level of 11.6 years and mean disease duration of 27.8 months. Most patients had mild to moderate disability (mean ALSFRS-R 32.7). The following cognitive domains showed significant effect sizes compared to controls (Hedges' g): social cognition (0.78); delayed verbal memory (0.62); fluency (0.60); visuoconstructive functions (0.59); global cognition (0.59); language (0.57); immediate verbal memory (0.56); executive functions (0.46); attention (0.43); psychomotor speed (0.42); and visual memory (0.27).

Discussion and conclusion: A new cognitive domain, that is social cognition, showed the largest effect size. Although this finding should be interpreted with caution, as only three studies included social cognition measures, it highlights the

clinical overlap between ALS and frontotemporal dementia. The update further reinforces the view that the cognitive profile extends beyond executive dysfunction and includes language and memory impairment (verbal > visual). In the visuoconstructive and psychomotor domains bias due to motor impairment could not be ruled out. Based on these findings, we propose to cover social cognition, verbal memory, fluency and language in a cognitive screen, which will include the following tests: Faux Pas Recognition Test, Rey Auditory Verbal Learning Test, Verbal Fluency (Letter; fluency index), and the Boston Naming Test. These tests are readily available and can be adjusted to correct for motor impairment or dysarthria.

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P61   THE VERBAL FLUENCY INDEX: NORMATIVE DATA BASED ON A SAMPLE OF HEALTHY DUTCH CONTROLS

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Keywords: fluency, VFI, normative data

Background: Cognitive changes have been demonstrated in 30% of ALS patients with verbal fluency being the most consistent deficit. For ALS patients, the verbal fluency index (VFI) has been developed which corrects for speech and motor impairment (1). The absence of normative data for the VFI hinders the interpretation of fluency deficits in individual patients and restricts the use of the VFI as a marker of cognitive performance.

Objective: To provide normative data for the 3-minute verbal fluency index based on a sample of age-matched healthy controls.

Methods: Three hundred and eighty-three native Dutch speaking volunteers were randomly selected by general practitioners who look after patients with ALS participating in a population-based study aiming at complete ascertainment of all patients with ALS in the Netherlands. The volunteers were age-matched with ALS patients and had no history of a neurological or psychiatric disorder or previous significant head injury. We recorded age, gender, education, medication, past medical history and use of toxic substances. The VFI, a 3-minute letter fluency task adjusted for motor impairment, was administered according to standard procedures. We used the letter "D" as this letter is comparable to the letter "S" in the English language, in terms of degrees of difficulty(2). Participants with more than five errors (perseverative errors, rule breaks and non-existing words) were excluded. To examine the effects of age, gender, education, medication, past medical history and use of toxic substances, a multiple stepwise linear regression was used.

Results: Three hundred and sixty-one participants (228 males and 133 females; mean age: 63; SD 9.5) were included. Educational level ranged from 'primary education' (4%) to 'academic/college degree' (27%) and was inversely correlated ($p < 10^{-7}$) to VFI performance. The VFI scores did not relate to the other variables. The regression formula to transform raw scores into a standardized Z-score was: $Z = (11, 37 - (0.86 * education)) - VFI/3.44$.

Discussion and conclusion: In this large cohort of healthy volunteers, VFI scores were dependent on the level of education with higher scores in higher educated subjects, which is in agreement with standardised fluency tests. The educational level of our volunteer sample was comparable to a large Dutch ALS cohort. This normative data can be used to quantify fluency impairment in individual Dutch ALS patients and thus serve as a marker of cognitive impairment in ALS. Validation of these findings warrants similar studies in other countries.

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P62   BRAIN COMPUTER INTERFACE AND EYE-TRACKING FOR COGNITIVE ASSESSMENT IN AMYOTROPHIC LATERAL SCLEROSIS: THE EBRAIN PROJECT

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Keywords: eye-tracking, brain computer interface, neuropsychological assessment

Background: Many patients affected by amyotrophic lateral sclerosis (ALS) show cognitive alterations, especially regarding frontal executive functions, ranging from a mild cognitive impairment to frontotemporal dementia clinical profiles (1). Cognitive assessment is problematic in moderate-severe stages of ALS, due to the presence of motor-verbal impairment which makes the use of traditional paper and pencil tests poorly reliable or not feasible. Recently, Eye-tracking (ET) and Brain Computer Interface (BCI) have been preliminarily used in ALS to administrate cognitive testing (2–4). However, an extended motor-verbal free neuropsychological (NP) battery is not available for ALS longitudinal assessment.

Objectives: A recently funded project, 'eBrain: BCI-ET for ALS', aimed at evaluating the use of P300-based BCI and ET technologies to administrate cognitive tests in ALS.

Methods: Twenty-eight ALS patients (mean age: 62.6 ± 11.8; mean education: 9.6 ± 3.6) and 30 healthy subjects (mean age: 56.2 ± 11.9; mean education: 13.7 ± 4.2) underwent a traditional cognitive (*Frontal Assessment Battery – FAB*; *Montreal Cognitive Assessment – MOCA*) and psychological screening (*Beck Depression Inventory – BDI*; *State-Trait Anxiety Inventory – STAI – Y1,Y2*). Also behavioural features were recorded. Adapted versions of NP tests assessing verbal comprehension, frontal functioning, attentive and theory of mind abilities were administered with both BCI and ET. Furthermore, clinical data were collected (respiratory parameters and *ALS Functional Rating Scale – Revised – ALSFRS-R*) and usability of both devices was evaluated with an ad hoc questionnaire.

Results: Data showed significant differences between healthy subjects and ALS patients performances in BCI and ET adapted measures of frontal abilities ($p < 0.05$); furthermore, a correlation between traditional NP assessment and BCI-ET testing was found ($p < 0.05$), supporting the concurrent validity of the adapted measures. Finally, a better perceived usability was observed for ET overall, compared to BCI ($p < 0.05$); nevertheless, patients evaluated BCI as a more positive and useful tool in order to compensate the verbal limitation, in comparison to the evaluation given by controls.

Discussion and conclusion: These results support the good level of sensitivity of the BCI and ET-based NP assessment, offering promising insights into the use of such devices for the longitudinal cognitive testing in ALS. Besides, the high perceived usability not only of ET, but also of BCI system seems relevant, since BCI represents the only means to bypass verbal-motor deficit for patients in advanced stages of the disease, where ocular motility can also be damaged.

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P63　USE OF FNIR TO EXAMINE HEMODYNAMIC CHANGES DURING COGNITIVE TASKS IN INDIVIDUALS WITH ALS

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Keywords: functional near-infrared spectroscopy (fNIR), executive function

Background: Evidence suggests that deficits in frontally mediated executive skills occur in up to 50% of ALS patients (1). Dysexecutive and behavioural impairments are most common, and likely related to frontal lobe alterations (2,3).

Functional Near-Infrared Spectroscopy (fNIR) is a noninvasive optical neuroimaging technology that monitors hemodynamic changes in the brain, that is, blood oxygenation and volume, concurrently with cognitive tasks. Studies show that fNIR measures are sensitive to executive functions (4).

Objective: To examine and compare hemodynamic changes detected by fNIR during challenging cognitive tasks in ALS and healthy controls (HC).

Methods: Twenty-four subjects (11 ALS, ALSFRS-R: 36.8 ± 5.2) completed three neuropsychological tests: Philadelphia Brief Assessment of Cognition (PBAC), Mini Mental State Exam (MMSE), and Clock Drawing Test (CDT), and three fNIR-monitored tasks: 5-minute Psychomotor Vigilance Test (PVT), King-Device Test (KD), and Number Interference Task (NIT). The PVT, a sustained-attention, visual-response task, and KD, a rapid number naming task, were used to obtain baseline fNIR measures. The NIT, a computerized adaptation of the Stroop Test, triggered working memory and maintenance of a complex mental set. Demographics, medical history, ALSFRS-R, and pulmonary function tests were collected as applicable. Between group differences were assessed by Welch ANOVAs ($p = 0.10$) to account for unequal sample sizes and variances. Analysis focused on the NIT, which progresses through three trials of increasing difficulty. Correlations between mean oxygenation (μmolar) and clinical parameters were also examined.

Results: ALS subjects had more years of education ($HC = 13.3 \pm 1.8$, $ALS = 16.1 \pm 2.4$, $p = 0.004$), but no differences in PBAC, MMSE or CDT scores. Significant differences in oxygenation were found during NIT Trials 2 and 3 in the Right Dorsolateral Prefrontal Cortex (DLPFC) (Trial 2: $F_{(1,15.129)} = 4.03$, $p = 0.063$) ($HC = 0.393 \pm 0.385$, $ALS = 0.861 \pm 0.687$); Trial 3: $F_{(1,15.596)} 4.141$, $p = 0.059$) ($HC = 0.272 \pm 0.416$, $ALS = 0.766 \pm 0.707$)). Correlations were detected between oxygenation and PBAC subscales measuring Behavior/Comportment (Trial 2: Left DLPFC, Left/Right midPFC ($r = -0.367$ to -0.465, $p < 0.077$); Trial 3: Left midPFC, Right DLPFC ($r = -0.356$ to -0.424, $p < 0.088$)) and Executive functions (Trial 3: Right DLPFC ($r = 0.417$, $p = 0.043$)). Within the ALS group, ALSFRS Respiratory subscale correlated with oxygenation in the Left DLPFC and Right midPFC (Trial 3: $r = -0.591$ to -0.601, $p < 0.054$).

Discussion and conclusion: ALS subjects displayed higher oxygen utilization than HC to complete a challenging cognitive task, possibly indicating increased mental workload. Differences in oxygenation are noted despite lack of gross cognitive impairment, implying that fNIR may be more sensitive to early cognitive difficulties and frontal lobe involvement than neuropsychological testing alone. Furthermore, correlations between oxygenation and respiratory, executive and behavioral subscales imply that the fNIR is sensitive to changes in these at-risk areas.

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P64   THE ITALIAN VERSION OF THE ALS-COGNITIVE BEHAVIORAL SCREEN (ALS-CBS): A MOTOR NEURON DISEASE DEDICATED TOOL

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Keywords: screening test, cognitive dysfunction, ALS-CBS

Background: Cognitive/behavioral dysfunction has been recognized as a consistent part of the ALS clinical phenotype, ranging from a mild impairment to a full blown dementia. Currently used neuropsychological tools for exploring this novel clinical dimension often do not take into account physical disabilities of ALS patients, undoubtedly introducing a source of bias in the interpretation of results. The ALS-Cognitive Behavioral Screen (ALS-CBS) is a recently proposed ALS-dedicated tool separated into two subscales: a cognitive one (patient, max score 20) and a behavioral one (caregiver, max score 45).

Objectives: To test our Italian version of the ALS-CBS in a consecutive series of ALS outpatients, assessing its value for the screening of cognitive dysfunction.

Methods: Fifty-seven consecutive ALS non-demented outpatients were recruited (age: 65.2 ± 9.8 years old (range: 42–80); ALSFRS-R score: 31.7 ± 8.6). Each patient underwent the ALS-Cognitive Behavioral Screen (ALS-CBS), the Frontal Assessment Battery (FAB) and, due to previously shown FAB limits (Tremolizzo *et al* 2013), the Weigl's Sorting Test (WST).

Results: Mean ALS-CBS cognitive subscore was 13.2 ± 3.8 and a correlation with both FAB and WST raw scores was present ($r = 0.697$ and $r = 0.616$, respectively, $p < 0.0001$). Originally proposed (Woolley *et al.* 2010) cut-off values are less than 10 and less than 17 for dementia and mild cognitive impairment, respectively; however, most of our patients scored less than 17 (91.2%), and a consistent part less than 10 (17.5%). Possibly this data might in part be explained by the fact that mean education in our patients was 9.8 ± 3.9 years, lower with respect to the original ALS-CBS series, and both age and education correlated with the ALS-CBS cognitive subscore ($r = -0.400$ $p = 0.002$ and $r = 0.318$ $p = 0.016$, respectively). For this reason, we are currently testing healthy controls for producing opportune normative data.

The ALS-CBS behavioral subscores were obtained for 46 patients and mean value was 34.0 ± 9.2. With respect to original cut-off values, 16 patients (34.7%) scored less than 32, and 24 (47.8%) scored less than 36. Cognitive and behavioral score did not correlate between them or with the degree of motor impairment.

Discussion and conclusion: The ALS-CBS is a sensitive tool for screening cognitive and behavioral alterations in ALS. However, normative values are needed for improving its value.

P65   PRIMITIVE REFLEXES FOR THE SCREENING OF COGNITIVE DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: primitive reflexes, cognitive dysfunction, frontal assessment battery

Background: The presence of primitive reflexes (PRs) has been classically associated to cognitive dysfunction, albeit the role of these signs is highly debated since they are often present also in apparently healthy controls. Interestingly, the combination of more than one PR has been shown to have more discriminative value for the screening of cognitive dysfunction with respect to each single PR. Some PRs have been reported in amyotrophic lateral sclerosis (ALS) albeit never in relationship to the cognitive status; this question, however, might be of relevance since a continuum between ALS and frontotemporal dementia has already been postulated.

Objectives: Testing a battery of PRs including sucking, snout, grasping, Myerson's, palmomental and corneomandibular reflexes in ALS patients with respect to their cognitive status in an outpatient screening situation.

Methods: Fifty consecutive ALS outpatients were recruited (age: 65 ± 1.5 years old (range: 42–81); ALSFRS-R score: 29.7 ± 1.5); the putative presence of cognitive dysfunction was screened by the frontal assessment battery (FAB).

Results: The number of PR recorded in each patient (0–6) correlated with the FAB score ($r = -0.60$, $p < 0.0001$) and dichotomizing patients for presence versus absence of cognitive dysfunction (FAB score < 13.4) the number of PR was 2.8 ± 0.3 ($n = 14$) versus 1.3 ± 0.1 ($n = 36$), respectively ($p = 0.0003$). Only Myerson's, palmomental and corneomandibular reflexes were consistently prevalent in our population (45–65%), while the other three PR were under-represented.

Discussion and conclusion: According to our results, PR might give useful information regarding the frontal function of ALS patients in an outpatient setting. Albeit each single PR might lack of specificity, the added value of an array of PRs emerges. Hence, including a battery of selected PR in the neurological examination might be of value for the fast screening of cognitive dysfunction in ALS.

P66 CALLOSAL DYSFUNCTION AND COGNITIVE IMPAIRMENT IN ALS: IS THERE A LINK?

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Keywords: corpus callosum, cognition, frontal lobe

Background: In ALS, neuroimaging studies have demonstrated a prominent involvement of white matter, encompassing, as key structures, not only the corticospinal tract but and the corpus callosum(CC)(1). The observation of mirror movements was the first evidence of callosal impairment in interhemispheric inhibition (2). Although myelination breakdown of the callosal motor segment was found to be unrelated to the neuropsychological performance (3), cognitive decline psychiatric symptoms have been found in ALS patients showing substantial atrophy of the anterior fourth of the CC (4).

Objectives: We aimed to determine callosal dysfunction (CD) in ALS, on the basis of performances of task evaluating the integrity of the sensory-motor segment of the CC (s-mCD) and of novel tasks assessing bimanual motor control (BMC), and its correlations with clinical, cognitive and behavioural profiles.

Methods: Twenty-six non-demented ALS patients were enrolled after neurological evaluations. Integrity of s-mCD was quantified with tasks assessing the ability in interhemispheric transfer of motor-inhibitory input (3) and somesthetic information (5). Control of inhibition and interference during bimanual complex movements were assessed with newly developed tasks. An in-depth neuropsychological test battery was used to evaluate executive functions, social cognition, memory, attention, language and visual-spatial skills. Behavioural dysfunction was assessed through caregiver interviews. Cluster and correlations analyses between CD measures and neuropsychological data were computed.

Results: Patients with high s-mCD scores obtained lower performances on verbal fluency and verbal episodic memory tests. The novel CD measure was strongly related to executive functions (verbal fluency, control of inhibition and interference), frontally mediated aspects of memory (working memory, short-term memory and false recognitions) and social cognition (emotion recognition, interpersonal reactivity index). A cluster analysis considering as independent variable the new CD measure discriminated two groups of patients according to the degree of cognitive impairment quantified as the percentage of cognitive performances below the normal range. Overall CD measures did not correlate with clinical signs of ALS.

Discussion and conclusion: We demonstrated that higher CD values corresponded to lower neuropsychological performances, suggesting a correlation between CD and cognition in ALS. In particular, we developed novel tasks to assess BMC, which were strongly associated with frontal function. We speculate that correct performances in BMC tasks need the integrity of the anterior portion of the CC connecting dorsolateral prefrontal cortex and cingulate cortex in order to prevent interference from the opposite hemisphere. More generally, CC impairment might interfere with neuronal circuitry responsible of executive and behaviour control. Future studies are warranted to investigate if the involvement of the anterior portion of the CC can predict cognitive decline in ALS.

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P67 PATTERN OF PERSONALITY CHANGES IN ALS: A PRELIMINARY LONGITUDINAL STUDY

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Keywords: personality, neuropsychiatry, cognition

Background: While personality is a complex and only partially understood construct, different theoretical models allow the inter-individual differences as well as intra-individual longitudinal changes in personality to be quantified. Currently, one of the more widely used models of personality is represented by the "five factors" personality model (1). According to this model, personality results from the stable balance of five key traits, extraversion, agreeableness, conscientiousness, neuroticism and openness to new experiences.

While pervasive personality changes represent one of the key features of different neurodegenerative conditions (2), the presence and extent of personality alterations in ALS are only partially understood.

Aim: Aim of the study was thus to longitudinally evaluate personality traits according to the five factor model in a group of ALS patients to explore the presence of personality changes over time and as well as the cognitive and behavioural correlates.

Methods: Twenty-two subjects with a diagnosis of definite or probable ALS were included in the study. A control group of age- and sex-matched healthy subjects was also included in the study. All patients underwent clinical, cognitive and behavioural evaluations at baseline and at follow-up after 6 months. In both ALS patients and controls, personality was evaluated at both time points with the Big Five Inventory (3) using self-report and observer-based questionnaires.

Results: Compared to controls, ALS subjects presented at baseline with reduced agreeableness and conscientiousness scores as evaluated with the observer-based questionnaire. Moreover at the follow-up evaluation, ALS subjects presented with a further reduction in both agreeableness and conscientiousness scores compared to baseline. Lastly agreeableness and conscientiousness scores at baseline were respectively associated with increased behavioural disturbances and cognitive impairment at follow-up.

Discussion: Here, we showed a reduction of agreeableness and conscientiousness scores in ALS subjects both compared to controls and over time. Moreover development of cognitive or behavioural disturbances over time was correlated with more pathological agreeableness and conscientiousness scores at baseline. Given the known relationship between prefrontal structural alterations and the two aforementioned personality traits (4) our observation is in line with the known involvement of prefrontal areas in ALS. Our data suggest expansion of the current construct of non-motor facets of ALS to include personality alterations and suggest that personality assessment could provide useful information to the plan of ALS patients.

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P68   CHARACTERISTICS AND CLINICAL SIGNIFICANCE OF EXECUTIVE FUNCTIONING IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) WITH AND WITHOUT FTD

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Keywords: executive functioning, neuropsychology, cognition

Background: Executive dysfunctions are a key indicator for a negative prognosis in ALS (Elamin, 2011). Symptoms closely related to executive functioning are interconnected with behavioural abnormalities, for example, apathy, disinhibiton, and disorganization. Both behavioural abnormalities and cognitive impairments are poorly reflected at the clinical level though they are highly prevalent when assessed by neuropsychological measurements.

Objectives: The aim was to investigate executive dysfunctions in relation to their relevance in everyday functioning.

Methods: We conducted a prospective neuropsychological study of 103 patients with ALS (including 11 ALS-FTD cases) and 71 age, education and intelligence matched healthy controls. We used a comprehensive neuropsychological test battery adapted to motor incapabilities, including a behavioural questionnaire. All executive test variables were assigned to their corresponding level of cognitive regulation (basic,

complex, social and emotional). Previous criteria according to Strong (2009) and Phukan (2012) were modified, and all patients were categorized regarding cognitive and behavioural abnormalities. By analyzing frequency distributions, comparisons between cognitive subgroups at all levels of cognitive regulatory processes were performed.

Results: 19% of ALS patients without FTD and all ALS-FTD patients demonstrated executive function impairment. The extent of basic function disorders (eg shifting, updating, and fluency) ranged from 28% to 78% in patients without FTD, from 45% to 100% in ALS-FTD patients. In contrast, impairment of complex functioning (eg monitoring, problem solving) was not particularly prevalent regardless of FTD (27%). Difficulties in problem solving occurred even only in ALS-FTD cases. Behavioural disorders were apparent in 16% of ALS patients and 54% of ALS-FTD patients.

Discussion and conclusion: ALS patients with executive function impairment had a significantly higher frequency of impaired basal regulatory processes in contrast to more complex processes or rather impairments at the highest level of executive functioning (activity and social behaviour). Processes controlling behaviour, however, seem to be only affected in ALS-FTD. Basal executive functions are less relevant to everyday functioning than complex functions such as problem solving. This may explain the lack of relevant abnormalities in ALS patients based on our clinical observation. Our results indicate a pattern of executive impairment in ALS patients without FTD which appears to be not as severe to affect everyday's functioning.

P69   SEMANTIC IMPAIRMENT PROFILES OF THE FTD-MND CONTINUUM

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Keywords: FTD-MND continuum; cognitive impairment; semantic

Background: Increasingly, cognitive and behavioural deficits are recognized in Motor Neuron Disease (MND), and a proportion of patients develop frank frontotemporal dementia (FTD-MND). Executive impairment is well characterized in MND, but non-executive deficits have not been investigated in detail. Aphasia is characteristic of some FTD phenotypes; for example, semantic deficits characterize the semantic dementia (SD) phenotype of FTD. Nonetheless, the nature and extent of semantic impairment in MND remains unexplored, even though recent studies have described SD-like anterior temporal lobe changes in the disease.

Objectives: The current study aimed to compare semantic deficits in MND and FTD-MND, compared to those seen in SD and controls.

Method: Semantics were evaluated using the Sydney Language Battery (SYDBAT), a test of single-word processing

comprising four subtests: three specifically designed to assess semantics (confrontational naming, comprehension, and semantic association).

Results: In total 112 participants were recruited from a multidisciplinary research clinic in Sydney (mean age = 64.9; male = 63.4%; mean years of education = 12.7); 24 patients with MND, 26 patients with FTD-MND, 33 patients with SD and 29 age- and education-matched healthy controls. Significant semantic impairments were observed in MND and FTD-MND, compared to controls. The MND cohort demonstrated the mildest impairment for both confrontational naming and word comprehension, although performed comparably to controls for semantic association. The FTD-MND group performed significantly below MND participants on all semantic subtests, but not as poorly as the SD group.

Discussion: The present study demonstrates that semantic impairment is present in MND and FTD-MND. The severity of this disturbance however differs, with a mild impairment being observed in MND patients and a significantly poorer performance by FTD-MND patients, albeit not as severe as in SD. The increasing severity of semantic impairment across the three patient groups supports the view of an FTD-MND continuum. Importantly, these findings implicate left anterior temporal lobe involvement, which is acknowledged as the semantic hub and critical in linking representations across cortical regions. This is consistent with imaging studies, which have reported changes to anterior temporal regions in both MND and particularly in FTD-MND.

Conclusion: Semantic deficits may be a feature of MND and FTD-MND, potentially reflecting left anterior temporal lobe involvement. These findings highlight the necessity for clinical assessment of semantic knowledge in MND, not only to ensure optimal patient communication, but also to detect non-executive cognitive impairment.

P70   CEREBROSPINAL FLUID PROGRANULIN LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS ARE ASSOCIATED WITH FRONTO-EXECUTIVE DYSFUNCTION

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Keywords: progranulin; cerebrospinal fluid; fronto-executive dysfunction

Background: There is an overlap between fronto-temporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) clinically and genetically. Mutation of the progranulin (GRN) gene causes FTLD and less frequently ALS. GRN can be measured in serum and cerebrospinal fluid (CSF) and was found to be decreased in FTLD. In ALS patients CSF GRN showed an inverse correlation with disease duration.

Methods: CSF analysis including GRN and extensive neuropsychological examination was applied to healthy controls and ALS patients. Furthermore, the GRN levels in CSF of ALS patients were compared to FTLD and control subjects.

Results: Included were 19 healthy controls (age: 66.8 ± 6.8 years) and 30 ALS patients (age: 62.8 ± 12.6 years). For CSF analysis 38 patients with FTLD were examined (age: 68.6 ± 7.5). GRN in CSF was decreased in ALS (p = 0.03) and in FTLD (p = 0.003) compared to controls. In ALS subjects GRN was correlated to the MOCA as a summary score of cognition, to TMT-B and to verbal working memory, reflecting fronto-executive dysfunction.

Conclusion: In ALS patients we found abnormal levels of GRN in CSF. As in FLTD, GRN may serve as a biomarker indicating a cognitive impairment in ALS. Further study is necessary to determine the potential utility of GRN as a biomarker for frontal lobe degeneration in ALS.

P71   BEHAVIOURAL ASSESSMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: behavioural assessment, FrSBe, cognitive impairment

Background: Non-motor manifestations are subtly present in the vast majority of patients with amyothrophic lateral sclerosis (ALS), with a subgroup developing a frontotemporal dementia (FTD). The presence of behavioural or dysexecutive dysfunction is a negative prognostic indicator and patients with ALS-FTD have a shorter survival than those with classic ALS.

Objectives: To evaluate the frequency of neurobehavioral symptoms related to FTD, to determine the relevance of changes and to ascertain the relationship between cognitive and behavioural changes.

Material and methods: Two hundred and two ALS incident cases were assessed for the presence of cognitive and behavioural aspects. Different domains were considered (attention, memory, language and visuo-praxic abilities); behavioural abnormalities were assessed with the FrSBe. Cognitive impairment was defined when scores were equal or below the 5th percentile, compared to age- and education-matched norms. Binary correlation was evaluated with Pearson's coefficient. Differences between patients and caregivers were tested (paired t-test and Cohen's K coefficient). FrSBe scores were included as T-scores.

Results: A group of 183 patients (19 excluded): 177 Male and 77 Female; mean age of 67.3 (± 9.7) years; mean educational level of 8.3 (± 4.1) years; mean duration of the illness of 15.4 months (± 13.5), was considered for statistical analysis.

In 23 cases (12.6%) a diagnosis of possible or probable dementia of the frontotemporal type was defined.

According to the cut-off, more than one-third exhibited behavioural impairment on the FrSBe "after" total scale rated by caregivers (41.5%) with a predominance of Apathy (51.8%), followed by Executive impairment (25.6%) and Disinhibition (25.6%). Neurobehavioral symptoms were not related to patients' age and gender, to the duration of the disease, to bulbar onset, or to the presence of bulbar symptoms at the time of the interview. They were instead significantly related to the physical status ($p < 0.05$). Considering delta scores, 13% of self-administered and 28% of caregivers' questionnaires reported relevant changes at the 'present time'.

For a group of 125 patients' and caregivers' coupled questionnaires were examined: in most cases (46.1%, n = 53) patients and caregivers shared the opinion about the absence of pathology (Cohen's K = 72); in nine cases (7.8%) patients agreed on the presence of a behavioural disturbance, in three cases (2.6%) having a score above the cut-off. Patients with impaired cognition had greater rates of behavioural impairment.

Discussion: Results at the FrSBe can be different when considering the 'after' scores (caregivers' forms T-scores) or distances resulting in comparing the pre-symptomatic and the disease phase, highlighting phenocopy cases. We observed a rating correspondence between patients and caregivers and a concordance regarding the overall condition of absence/presence of behavioural impairment. As expected, this correlation was not present in patients with high levels of cognitive/behavioural pathology.

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P72　A NOVEL TOOL FOR DETECTION OF NEUROPSYCHIATRIC SYMPTOMS IN ALS – THE MIND-BEHAVIOURAL (MIND-B)

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Keywords: *neuropsychiatric symptoms; behavioural changes; assessment*

Background: There is great need for short, reliable and valid assessments of neuropsychiatric symptoms in ALS. Apathy is pervasive in ALS, but other challenging symptoms such as disinhibition and stereotypical behaviour have also been recognised. Crucially, neuropsychiatric symptoms have been shown to explain high levels of carer burden in ALS.

Objective: (1) To develop a novel tool to detect neuropsychiatric symptoms in ALS; (2) to compare levels of apathy, disinhibition and stereotypical behaviour in ALS; (3) to compare ALS and ALS plus patients in the novel tool.

Methods: One hundred and forty patients (Limb onset = 74.3%; bulbar onset = 25.7%) were included in the development of the novel tool, MiND-B, which is a reduced version of the Cambridge Behavioural Inventory (CBI-R). Rasch analysis was employed to identify the minimum amount of questions, and which were the best questions from the CBI-R that described the behavioural impairment in the ALS sample. The MiND-B contains 12 questions assessing neuropsychiatric symptoms, with Likert scores designed in a similar fashion to the ALSFRS-R to facilitate use and face validity; higher scores reflect normal behaviour. The MiND-B demonstrated construct validity (Mean infit and outfit statistics = 1.05; Z = 0.2, SD = 0.19); good reliability (Cronbach alpha = 0.97), and unidimensionality (raw variance explained was 44.4%). The MiND-B can be completed by the informant.

An independent sample of 79 patients was used for the validation analysis. Limb (70%) and bulbar onset patients (30%) were compared in their MiND-B scores. ALS plus and ALS patients (40/79), subcategorised according to the Strong criteria (2009), were also compared in their MiND-B scores. All patients were diagnosed following current criteria for ALS. Non-parametric tests were used for the comparison of symptoms and disease subgroups.

Results: Limb and bulbar onset patients did not differ in levels of apathy, disinhibition or stereotypical behaviour (p > 0.05). When comparing types of behaviour, however, apathy was the most marked neuropsychiatric symptom, followed by stereotypical behaviour and disinhibition (p < 0.05). Critically, the MiND-B could differentiate patients subcategorised into ALS or ALS plus on disinhibition and stereotypical behaviour (p < 0.05). Apathy was pervasive.

Discussion: The MiND-B has demonstrated all necessary psychometric properties as a valid, reliable and unidimensional tool to detect neuropsychiatric symptoms in ALS. It can differentiate types of behavioural symptoms, and more importantly, can also help in differentiating patients with ALS plus from those who present with pure motor symptoms.

Conclusions: The MiND-B is a useful, short, valid and reliable tool for use in ALS clinics and research studies. It is available from the authors and free of charge.

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P73　DELAY IN DIAGNOSIS IS A MAIN RISK FACTOR FOR DEPRESSION IN MND

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Keywords: *depression, delay in diagnosis, psychological interventions*

Background: Despite severe physical impairment, patients with MND report satisfactory quality of life (1). Accordingly, the prevalence of major depression among these patients is relatively low contrary to what people may expect (2). This is likely to be due to unique risk factors underlying depression in this patient population, which remain unclear. Clarifying predisposing and precipitating factors related to depression in MND can help with identifying patients at risk of developing major depression and establish specific goals of psychological intervention.

Objective: To identify risk factors for depression in MND patients.

Methods: Forty-one patients with MND (61% female; 61% limb onset; mean age = 64.5 years, $SD = 12$) completed questionnaires on medical and psychological history, family background and depression (Depression, Anxiety, Stress Scale-21). Patients' level of functioning was assessed by the ALSFRS-R. Direct logistic regression was performed to assess the impact of various factors (physical function, time between symptom onset and diagnosis, family and personal history of mental illness) on the likelihood that patients would report a high level of depression.

Results: The average time between symptom onset and diagnosis was 15.8 months ($SD = 13.7$). ALSFRS-R scores between limb and bulbar onset patients were similar ($p = 0.54$). Depression scores did not vary across patients with limb or bulbar onset MND ($p = 0.49$). A similar proportion of patients reported a family (32%) or personal (29%) history of mental illness (12% had both family and personal history of mental illness). Most patients reported no depressive symptoms (66%). The logistic regression revealed that only delay between symptom onset and diagnosis predicted the likelihood of reporting high levels of depression ($p < 0.05$).

Discussion: The findings of this study suggest that delay between symptom onset and diagnosis is a key risk factor for depression in MND. Patients who experienced a longer delay in diagnosis were more likely to report depression and are at higher risk of developing more severe symptoms. These patients may be prime targets of psychological intervention. The findings also suggest that interventions focusing on helping patients cope with the process of differential diagnosis may be particularly useful, especially given that there are no specific tests for MND. Often, the remaining confirmatory factor for the diagnosis is symptom progression. Identifying and supporting patients at risk of developing major depression ensures cost-effective and evidence-based psychological intervention.

Conclusion: Understanding disease-specific risk factors for depression in MND is important for developing tailored psychological interventions. This study suggests that delay in diagnosis is a key precipitant of depression in MND. Specific psychological approaches are required to take into account patients' experiences from symptom onset to definitive diagnosis of MND.

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P74   CONFOUNDERS OF DEPRESSION MEASUREMENT IN ALS/MND: META-REGRESSION ANALYSIS OF PUBLISHED LITERATURE

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Objective: To estimate measurement bias from popular questionnaire measures for depression using meta-regression.

Methods: A systematic review of the literature published on the following databases was conducted without time limits: PubMed (MEDLINE), PsycINFO, the Cochrane Central Register of Controlled Trials, CINAHL, Scopus and ISI Web of Science. Mean scores for depression questionnaires were separately meta-regressed on study-level mean times since onset of ALS/MND symptoms in order to test the significance of the slopes and assess the goodness of fit of the two conditional random models.

Results: Our literature search revealed 110 studies that reported depression scores from psychometric questionnaires. Data were included from 103 studies (3,190 patients). The most commonly used questionnaires were The Hospital Anxiety and Depression Scale (HADS) and the Beck Depression Inventory (both the BDI-I and the BDI-II).

Average scores on both questionnaires do not indicate a large number of patients with case-level depression and average scores for depression are similar to score from normal populations. Scores for depression increase with duration of illness. The BDI and the BDI-II were highly influenced (more than 50% of the variance in scores) by level of physical impairment. Scores from the HADS were also influenced by physical impairment, but to a lesser degree (19%).

Discussion: Meta-evidence from the published literature suggests that commonly used generic depression scales are overly influenced by the presence of impairment.

Conclusion: Widely used depression scales over-estimate severity of depression due to confounding with physical symptoms of the underlying physical symptoms in ALS/MND.

P75   GROUP INTERVENTION BASED ON MINDFULNESS FOR HOSPITAL HEALTH CARE PROVIDERS: TAKING CARE OF THOSE WHO CARE

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Background: Stress and compassion fatigue is a common part of everyday life in neuromuscular clinics. These degenerative conditions are highly stressful not only for patients and caregivers but also for health care providers, usually living

with pain and death. The greatest risk for health care providers is to develop burn-out syndromes which imply reduced emotional involvement and respect for other people negatively affecting relationships (1). Meditative practice, based on Mindfulness Training developed by John Kabat Zinn (MBSR) is quickly spreading in the health field (2). This program has proven successful to reduce stress levels and promote skills inherent in the concept of resilience (3).

Objective: To promote group interventions to prevent burn-out and relieve the distress of healthcare professionals and also to provide useful tools to manage stressful situations and improve personal skills to manage stress and pain.

Methods: This group intervention consists of five session of one hour each. Five health care providers are involved in each group, starting with nurses and physiotherapists. By working with a meditative instructor and a psychologist health providers have the opportunity to cope with their feelings and exercise with some meditation practice at their workplace. Meditation practices combined with cognitive restructuring help to develop a new point of view that allows individuals to cope with negative and painful experience in a non-judgmental and accepting manner.

Results: We expect that the implementation of this group in the workplace can lead to: reduced burn-out symptoms, both physical and psychological; increase quality of life perception and life satisfaction, reduced levels of stress and negative thinking (rumination). These outcomes will be evaluated by structured and validated tests.

Discussion and conclusion: If Mindfulness combined with cognitive psychological approaches proves to reduce the risk of burn-out, we recommend that this intervention is included in clinical practice to enable health care providers to better manage their stress and develop their own coping strategies.

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P76   ALS PATIENT'S DEATH: PSYCHOLOGICAL IMPACT ON THE CAREGIVER

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Keywords: caregiver, complicated grief, attachment style

Background: Caring for ALS patients during the disease proved to be very demanding in terms of anxiety, depression, and somatic symptomatology and to cause a poor quality of life. However, there is only little knowledge on the psychological impact of surviving the death of an ALS patient on family caregivers (1,2).

Objectives: The main objectives were to investigate the psychological impact of the death of patients with ALS on their caregivers, in terms of personality characteristics, mood tone, the implicit relational style (attachment style), quality of life and complicated grief disorder (CG) (3), as defined by new DSM-V manual criteria. Understanding the psychological consequences of ALS patients' death can help clinicians to identify those caregivers who are likely to experience more severe psychopathological consequences of bereavement.

Methods: Forty-six family caregivers of deceased ALS patients participated in the study. Participants underwent an in-depth telephonic interview about their experience and were asked to complete questionnaires orally proposed: the Relationship Questionnaire to assess adult attachment; the short-form revised Eysenck Personality Inventory, to measure personality traits; the Parental Bonding Instrument which measures subjectively perceived parental characteristics; the Inventory of Complicated Grief to assess symptoms of complicated grief; the Life satisfaction Index, short form to evaluate the mood tone and the quality and life satisfaction of caregivers.

Results: Main results revealed that 17 participants (39%) met the DSM-V criteria for CG. Furthermore only a minority of the caregivers showed a secure adult attachment (about 22% of the total); the great majority of participants were insecure (about 78%), with a prevalence of the dismissing attachment style (63%).

Discussion and conclusion: The preliminary results of the study showed that the loss of a familial ALS patient may have dramatic psychological consequences on their caregivers in terms of attachment relational representation and CG. Only a small percentage of the caregivers had a secure attachment style, a percentage significantly lower than the normative population. Since attachment relational style is considered a stable psychological trait negatively influenced only by catastrophic emotional events, this finding appears as particularly relevant. Analogously, a relevant proportion of the caregivers of deceased ALS patients met the new criteria for Complicated Grief such as feelings of disbelief, being stunned, avoidance, anger, shock, and separation distress (ie yearning and searching for the deceased, excessive loneliness, personal guilt, auditory and visual hallucinations). Further analysis is warranted to identify predictors of complicated grief.

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THEME 4 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

RIGHTSLINK

THEME 4 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P77   NEUROIMAGING IN ALS AS A FEASIBLE STRATEGY FOR CLINICAL THERAPY: TARGETS AND LIMITS

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Keywords: SPIOn, migration, stem cell

Background: In the absence of effective pharmacological treatments for amyotrophic lateral sclerosis (ALS), a novel promising therapeutic strategy resides in stem cell (SC) transplantation for the replacement and/or protection of damaged neurons. An important, still unsolved, challenge is the imaging of SCs to monitor their fate and interactions with host tissue after transplantation. A common technique consists of cell labelling with small paramagnetic iron oxide nanoparticles (SPIOn), detectable by magnetic resonance imaging (MRI).

Objectives: The aims of the present study were to monitor the advantages and enlighten the limits of the clinically approved SPIOn labelling from *in vitro* to *in vivo*, in the pathological Wobbler mice, a well-characterized ALS animal model.

Methods: Nanoparticles, composed by carboxydextran and paramagnetic iron oxide, were loaded in human foetal SCs for 72 hours in conjunction with a suitable live cell DNA dye. SCs were labelled with SPIOn and their motility was evaluated by a specific cell migration assay. Thereafter, cells were intracerebroventricularly injected in Wobbler mice and monitored at different times (24h, 2, 6 and 8 weeks after graft).

Results: We observe that the double labelling did not interfere with cell proliferation and survival. However, we also found that SPIOn labelling significantly reduce SC migration. Motility impairment was directly proportional to loaded SPIOn amount while the migration rate of labelled cells was only partially restored by a degenerative surrounding. After injection in mice, MRI analysis showed a consistent signal confined in the whole ventricular compartments until the 2nd week after graft. In the following 6 and 8 weeks it decreased, but remained still visible in the same regions. *Ex vivo* studies confirmed that the MRI signal overlapped with Hoechst 33258 labelling in mice without relevant differences.

Discussion and conclusion: The overall results obtained revealed that (1) biological properties of SCs resulted unaffected by double labelling; (2) SPIOn labelling could affect cell motility in a dose-dependent manner, shedding the light on another important parameter that could be crucial for the creation of novel protocols applicable to clinic; (3) an ongoing degenerative environment could partially restore normal motility; (4) *in vivo*, transplanted cells diffused in the whole ventricular system with a reduced, but still detectable, MRI signal over time; and (5) the macroscopic localization of SCs appeared not influenced by the pathological state affecting Wobbler mice.

Altogether, our results demonstrate that establishment of an optimal SPIOn dose, enabling both an excellent visualization of grafted cell by imaging and the maintenance of migration ability, appears fundamental to improve the effects of SC therapy. Alternative dyes (ie genetic or near infrared emitting tracers) should be investigated in pre-clinical settings as they may be more suitable for the future clinical treatments of ALS patients.

P78   NEUROIMAGING CORRELATES OF IMPAIRED MOTOR FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: fMRI, VBM, biomarker

Background: Neuroimaging studies in patients with ALS have shown differences in brain structure and function compared to healthy subjects. It is, however, not known whether and to what extent these changes relate to the functional measures of the disease.

Objectives: To correlate structural and functional magnetic resonance imaging data to functional measures of the disease.

Methods: Functional magnetic resonance imaging (fMRI) and voxel-based morphometry (VBM) were performed in eight ALS patients (age range: 46–82 years) on a Siemens Trio 3T scanner. For fMRI, a visuo-motor handgrip task at the 30% of the individual maximum force was used. Image pre-processing and statistical analysis was done using SPM8. A second level random effects analysis was used with a cluster-level significance $p < 0.05$ (FDR-corrected). A range of disease measures was tested in regression models: age, disease duration, ALS Functional Rating Scale (ALS-FRS), handgrip strength, upper motor neuron burden and compound muscle action potential (CMAP) amplitude.

Results: Group fMRI activations included contralateral primary sensorimotor cortex, lateral premotor cortex, posterior parietal cortex and supplementary motor area. Disease duration, ALS-FRS, handgrip strength and CMAP amplitude showed some statistically significant correlations with fMRI activity. There was no effect of age and upper motor neuron burden on fMRI activations. VBM measures (cortical grey matter density) correlated with ALS-FRS and not with other functional measures.

Conclusions: Cortical fMRI activity in ALS patients correlates to a certain extent with some of the disease measures. In general, fMRI activations decreased with disease duration and loss of function. Structural cortical changes showed only weak correlation with disease measures. Using functional and structural neuroimaging as a biomarker for ALS disease progression seems a possibility but there are some methodological issues to be considered. Longitudinal studies of larger groups of patients at different stages of the disease are warranted.

P79   TRACKING DISEASE PROGRESSION BY MRI: CLINICAL FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS CORRELATE WITH FOCAL CORTICAL THINNING OF THE MOTOR CORTEX

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Background and objectives: Amyotrophic lateral sclerosis (ALS) is characterised by degeneration of upper (UMN) and lower motor neurons (LMN). A tool to objectively quantify disease pathology in the brain would serve as both a surrogate endpoint and as a risk marker for future clinical trials. The aim of the present study was to relate clinical variables to cortical thinning of the primary motor cortex (PMC) measured by magnetic resonance imaging (MRI).

Methods: The PMC was defined as region of interest in high-resolution structural MRI scans. We related vertex-wise measures of cortical thinning to UMN involvement, bulbar/limb onset, the total ALS functional rating scale (ALSFRS-R), and its bulbar and upper limb subscore. In total, 93 ALS patients were recruited, 60 classical ALS, 17 with UMN dominant ALS, and 16 with pure LMN dominant ALS variants and compared to 67 age- and gender-matched healthy controls. The analyses were then repeated for the average cortical thickness of the PMC.

Results: Vertex-wise analysis revealed bilateral thinning of bulbar regions of the motor homunculus when UMN signs were found at the brainstem level, and in hand and arm regions when UMN signs were detected at the spinal level. The site of disease onset (bulbar/limb) exhibited the most pronounced thinning in the corresponding part of the motor cortex. UMN dominant ALS patients demonstrated the most distinct thinning followed by classical ALS patients. LMN

dominant ALS variants did not differ from healthy controls. Analysing the average thickness confirmed these results. Moreover, the ALSFRS-R score correlated positively with thinning of the left superior PMC and the bulbar subscore with thinning of the bilaterally inferior PMC.

Discussion and conclusion: Focal morphological changes within the PMC correspond to clinically measured impairments and to the disease phenotype. Measuring cortical thickness may offer an objective *in vivo* marker of upper motor neuron involvement. Longitudinal data may clarify the sequential order of the clinically measurable changes and the morphological alterations in the brain, as described above.

P80   POOLING OF DTI METRICS DERIVED FROM DIFFERENT MR-PROTOCOLS: AN EX POST FACTO METHODOLOGICAL PILOT STUDY IN ALS

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Introduction: Diffusion tensor imaging (DTI)-based metrics are increasingly used for analyzing ALS associated white matter (WM) alterations. The objective of this retrospective study was to analyze if DTI-based metrics derived from different diffusion weighted magnetic resonance (MR) protocols could be pooled in order to obtain large study samples for DTI analysis at the group level in ALS.

Methods: Two-hundred DTI data sets from patients with ALS and controls were collected from the database of the Department of Neurology, University of Ulm. Data were recorded within 8 years between 2005 and 2013. In control data, statistical analysis of fractional anisotropy, mean diffusivity, axial and radial diffusivity was performed in predefined regions of interests (ROIs), prone to be affected in ALS such as corticospinal tracts (CST) and corpus callosum (CC), together with regions that are probably not affected in ALS. Statistical comparison in terms of average FA-values and coefficients of variance (COV) (1) was performed for subgroups of control data that had been acquired by use of different MR-protocols, that is protocols with 13, 31 and 52 gradient directions, respectively. The same statistical methodology was then applied to the corresponding ALS patient subgroups. All analyses were performed by the Tensor Imaging and Fiber Tracking (TIFT) software (2).

Results: The statistical analyses of DTI-based metrics allowed to set up criteria for pooling DTI data that were derived from different MR-protocols. The criteria were based on F-statistics on averaged FA-values as well as on COV. Out of the total number of more than 200 DTI data sets greater than 70 % could be used for pooling and further analyses at the group level.

Conclusion: In summary, the retrospective statistical analysis was able to set up criteria for pooling of data that were recorded with different MR-protocols. The methodology is prone to be applied on the task of pooling multicenter DTI

data that usually include differences in MRI protocols due to local scanner-specific conditions.

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P81   DIFFUSION TENSOR TRACTOGRAPHY ANALYSIS OF THE CORPUS CALLOSAL FIBERS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: corpus callosum, diffusion tensor imaging, tractography

Background: Involvement of the corpus callosum (CC) was reported to be a consistent feature of amyotrophic lateral sclerosis (ALS). We examined the CC pathology using diffusion tensor tractography (DTT) analysis to identify the precise fiber bundles involved in ALS patients.

Methods: Diffusion tensor imaging was obtained from 14 sporadic ALS patients, and 16 age-matched, healthy controls. We performed whole brain tractography with multiple-ROI approach, and extracted CC fiber bundles in two different ways based on functional and structural relevance, that is, cortical ROI selection based on Brodmann area (BA), and on the sulcal-gyral pattern of cortical gray matter using FreeSurfer.

Results: The mean fractional anisotropy (FA) value was significantly reduced in ALS patients compared to controls in the callosal fibers interconnecting the primary motor (BA4), supplementary motor (BA6) and dorsolateral prefrontal cortex (BA9/46), but not the primary sensory cortex (BA 1, 2, 3), Broca area (BA 44/45) and orbitofrontal cortex (BA 11/47). The FreeSurfer ROI approach revealed very similar pattern of abnormalities. In addition, mean FA value of the callosal fibers interconnecting the primary motor area showed a significant correlation with disease severity, as assessed by revised Amyotrophic Lateral Sclerosis Functional Rating Scale, and clinical extent of upper motor neuron signs.

Conclusion: Our findings suggest that there may be some degree of selectivity or gradient in the CC pathology in ALS. The CC fibers interconnecting the primary motor and dorsolateral prefrontal cortex may be preferentially involved in ALS.

P82   PATH OF INTEREST-BASED DTI METRICS ANALYSIS ON AFFECTED TRACT STRUCTURES IN THE BRAINS OF ALS PATIENTS

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Keywords: diffusion tensor imaging, fiber tracking, individual level analysis

Introduction: In many studies, diffusion tensor imaging (DTI) has proven its applicability to identify ALS-associated patterns of brain alterations at the group level by voxel-wise comparison and whole brain-based spatial statistics (1). A different approach is the analysis of brain pathways identified by fiber tracking (FT) techniques (2). The objective of this study was to apply a new methodological FT-based approach to automatically analyze pathways that are prone to be involved in ALS by DTI-based FT.

Methods: From the database of the Department of Neurology, University of Ulmone-hundred and 26 DTI data sets from patients with ALS (N = 83) and controls (N = 53) investigated by the identical DTI acquisition protocol (1.5T) were analyzed using the following procedure. After stereotaxic normalization (under preservation of the intra-voxel directional information) of DTI data, starting seeds and target seeds for the FTs were defined. The structures to be analyzed included the corticospinal tract (CST) and other pathways known to become involved in the course of ALS. Out of all possible paths of the starting seed region, bundles of interest (BOIs) were defined as those paths that ended in the target seed region. The median path in each BOI was defined as path of interest (POI). The mean fractional anisotropy (FA) values underlying the defined BOIs and POIs were used for characterization of the pathway. All analyses were performed by the Tensor Imaging and Fiber Tracking (TIFT) software (3).

Results: The statistical analyses of BOIs and POIs allowed to automatically separate ALS and controls at p < 0.01 for various pathways.

Conclusion: The new technique of automatically defined BOIs and POIs at individual single subject level allows for individual analysis of predefined tract structures in neurodegenerative diseases with predefined affected WM structures such as ALS.

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P83　DISSOCIATION OF STRUCTURAL AND FUNCTIONAL MOTOR SYSTEM INTEGRITY ACROSS THE AMYOTROPHIC LATERAL SCLEROSIS – FRONTO-TEMPORAL DEMENTIA CONTINUUM

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Keywords: voxel-based morphometry, diffusion tensor imaging, threshold-tracking transcranial magnetic stimulation

Background: There is increasing evidence that amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) lie on a disease continuum. While cognitive deficits in ALS are increasingly recognised and shared with FTD, motor system dysfunction in the ALS-FTD continuum has been virtually unexplored.

Objective: To investigate the structural and functional motor system changes across the ALS-FTD continuum.

Methods: Seventy-nine individuals (ALS = 25, bvFTD = 17, controls = 37) participated in this study. Structural changes were assessed using a region of interest imaging approach of voxel-based morphometry (VBM) grey matter motor system regions and their diffusion tensor imaging (DTI) white matter connections. To elucidate the functional motor system changes, we conducted threshold-tracking transcranial magnetic stimulation (TMS) and measured neurophysiological index to measure both upper and lower motor neuron involvement. In a final analysis, we cross-correlated the structural and functional motor system data via covariation analyses. All results are reported at $p < 0.05$ corrected for family-wise error (FWE) or false discovery rate (FDR) multiple comparison correction.

Results: Structural results showed that in comparison to controls, ALS patients had substantially more white matter changes in corticospinal and motor cortical regions, while grey matter changes were mostly constrained to the cerebellum. BvFTD patients showed substantial grey and white matter changes across virtually all motor system regions, with the exception of the brainstem which was less affected than remaining regions. Direct comparison of ALS and bvFTD showed greater grey and white matter motor system changes in bvFTD compared to ALS, including in the motor cortex. By contrast, the functional motor systems integrity results revealed the opposite picture with ALS being worse affected than bvFTD and both patient groups showing increased excitability of upper motor neuron than controls. Cross-correlation analysis revealed that grey matter in motor cortical and cerebellar regions correlated with the excitability data for ALS, while bvFTD showed mostly supplementary motor and motor cortical areas related to these scores. Similarly, both ALS and bvFTD showed white matter integrity in the motor cortical areas covarying with TMS excitability, but bvFTD showed additionally more corticalspinal tract involvement on the level of the internal capsule while ALS showed corticalspinal involvement on the level of the brainstem.

Discussion: These findings reveal a dissociation of structural and functional motor system integrity across the ALS-FTD continuum, with bvFTD patients showing more structural motor system changes, while ALS having more functional motor system changes. Interestingly, cross-correlation of structural and functional data revealed further a neural dissociation of different motor system regions and tract covarying with the TMS excitability across both patient groups.

Conclusion: These findings suggest that structural and functional motor integrity measures do not converge for both pathologies, which will inform future diagnosis of motor system changes in both diseases.

P84　CORPUS CALLOSUM INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS: A PROBABILISTIC TRACTOGRAPHY STUDY USING Q-BALL IMAGING

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Keywords: fiber tracking, corpus callosum, q-ball imaging

Background: Diffusion tensor imaging (DTI)-based tractography has become a useful tool to investigate early corticospinal tract (CST) damage in amyotrophic lateral sclerosis (ALS). However, DTI model has shown some shortcomings in resolving individual fiber orientations within voxels containing fibers with more than one orientation. To overcome this limitation, fiber tracking packages that apply multi-tensorial algorithms, such as Q-ball imaging (QBI), have been proposed as effective alternative approaches.

Objectives: We explored WM tract abnormalities in phenotypically heterogeneous ALS cases aiming to establish a consistent WM signature of disease using QBI tracking algorithm.

Methods: Probabilistic tractography analysis was performed in 20 ALS patients (10 men and 10 women) and 20 age and sex-matched healthy controls. Diffusion-weighted datasets were loaded into QBI model using Diffusion Toolkit, a tool of Trackvis software package. Corpus callosum (CC) and CSTs were segmented in regions of interest (ROIs); genu, body and splenium for CC, and superior and inferior tracts for CSTs, from which WM tracts length (tl), fiber volume and density, and generalized fractional anisotropy (GFA) were extracted. Tractography parameters were correlated with clinical indices of pyramidal impairment (Upper Motor Neuron score), disease disability (ALS Functional Rating Scale-Revised) and progression (48-ALSFRS-R/months of disease duration).

Results: In ALS patients compared to controls, fiber density and volume were found significantly decreased, and tl significantly increased in all ROIs ($p < 0.05$, corrected). With regard to correlation analysis with clinical indices, in the body of CC pyramidal impairment was inversely related to fiber density ($p = 0.01$) and tl ($p = 0.05$). In the splenium of CC, clinical

disability (p = 0.013) and disease progression rate (p = 0.02) were inversely related to tl. In the superior tracts of left CST disease progression rate was inversely related to fiber density (p = 0.01).

Discussion and conclusion: Callosal involvement confirms to be a consistent feature of ALS pathology, significantly related to both upper motor neuron dysfunction and clinical disability. Specifically, structural damage of CC body might reflect pyramidal dysfunction, while structural changes of CC splenium may be associated with the grade of disease disability and progression. These findings indicate bilateral cortical impairment with an inter-hemispheric spread of the degenerative process. Moreover, the role of the increased fiber length observed in ALS patients might be attributable to compensatory mechanisms in response to impaired motor skills. This intriguing clue may provide further value to the hypothesis of structural brain plasticity triggered by the neurodegenerative process in ALS.

P85   RESTING STATE FUNCTIONAL CONNECTIVITY ALTERATIONS IN PRIMARY LATERAL SCLEROSIS

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Background: Like amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS) is a clinical diagnosis without established biomarkers. Analysis of resting state (RS) functional MRI (fMRI) reveals the temporal correlation between the low-frequency spontaneous fluctuations in the resting brain. These form functionally distinct networks that are related to specific sensory, motor, and cognitive brain functions. RS fMRI alterations have been observed in ALS patients.

Objective: To investigate RS functional connectivity of the sensorimotor and extramotor brain networks in patients with PLS, and to explore whether the RS functional connectivity alterations are related to patient clinical and cognitive status, and white matter (WM) tract damage.

Methods: RS functional MRI and diffusion tensor (DT) MRI were obtained from 24 PLS patients and 26 healthy controls. RS functional MRI was analyzed using independent component analysis and dual-regression technique. Using probabilistic tractography, the corticospinal tracts, corpus callosum (genu, body, splenium, and motor callosal fibers) and superior longitudinal fasciculi were obtained and tract mean FA values were calculated.

Results: Compared with controls, PLS patients showed an increased functional connectivity within the sensorimotor, frontal and left frontoparietal networks spanning the pre- and postcentral, medial and dorsal frontal, insular and superior temporal regions. Patients with more severe physical disability and more rapid rate of disease progression presented higher sensorimotor connectivity values. The increased functional connectivity within the frontal network was associated with

executive dysfunction. In addition, where the functional connectivity values were higher, greater was the structural damage to network-specific WM tracts.

Conclusions: This study shows clinically meaningful increased RS functional connectivity in PLS. Our findings raise the possibility that functional connectivity increases have a direct link with the pathogenesis of the disease.

P86   EXTRAMOTOR PATHOLOGY IS ASSOCIATED WITH COGNITIVE DEFICITS IN PRIMARY LATERAL SCLEROSIS PATIENTS

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Background: In early studies cognition was reported to be normal in primary lateral sclerosis (PLS). More recently, frontal lobe dysfunction syndrome in some PLS patients has been reported. Diffusion tensor (DT) MRI is currently unrivalled as a neuroimaging marker of upper motor neuron involvement and has the potential to provide an objective *in vivo* assessment of the extra-motor brain damage in motor neuron diseases.

Objective: The aim of this study was to determine the patterns of WM damage in PLS patients with cognitive impairment (PLS-ci) and those that are cognitively unimpaired (PLS-cu).

Methods: DT MRI scans were obtained from 26 PLS patients and 35 healthy controls (HC). All PLS patients underwent a neuropsychological battery of tests. The WM integrity was assessed using tract-based spatial statistics (TBSS) and the diffusion mean values were extracted from motor and extramotor WM tracts using probabilistic tractography.

Results: Ten PLS patients had abnormal scores in at least one neuropsychological test and were considered PLS-ci. Compared with HC, both groups of patients had a pattern of WM alterations in the corticospinal tract and body of the corpus callosum. Furthermore, compared with HC, PLS-ci patients showed an extra-motor damage involving the bilateral superior and inferior longitudinal fasciculi. The diffusion mean values of the extra-motor tracts were found to be altered in PLSci patients compared with both HC and PLS-cu groups.

Conclusions: This is the first study evaluating the relationship between cognitive performance and WM tract damage in PLS patients. PLS patients with abnormal cognition experience more extensive WM alterations than those with a preserved cognitive status. These results contribute to a better

understanding and characterization of extra-motor clinical manifestations in motor neuron diseases.

P87 CORTICAL MOTOR REPRESENTATION MAPPING BY NAVIGATED TRANSCRANIAL MAGNETIC STIMULATION AND VOXEL-BASED MORPHOMETRY. WHAT IS CLOSER TO THE TRUTH?

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Keywords: transcranial magnetic stimulation, voxel-based morphometry, brain mapping

Background: Navigated transcranial magnetic stimulation (nTMS) is a technique for the diagnosis of upper motor neuron function in ALS and *in vivo* motor areas of brain mapping. Another *in vivo* technique for gray matter atrophy detection is voxel-based morphometry (VBM). Both methods reflect the state of the gray matter: structurally (VBM), functionally (nTMS).

Objectives: To compare cortical motor areas and volume of gray matter measured by VBM.

Methods: The study involved 31 ALS patients (mean age 54.6 ± 12.2 years, ranging from 31 to 88 years old) and 24 healthy volunteers (mean age 39.5 ± 13.48 years). All respondents were right-handed. For ALS patients the median time from onset to inclusion in the study was 12 months. The patients' neurological status was evaluated using the revised ALS Functional Rating Scale (ALSFRS-R). The region of onset was bulbar in 12 patients, lower limb in 11 patients and upper limb in seven patients. Navigated TMS (NBS eXimia Nexstim) investigations including measurement of resting motor threshold and area maps size of m. abductor pollicis brevis (APB) cortical representation were performed for all participants. VBM was performed on ALS patients using 1.5 T Siemens Magnetom Avanto (3D T1 MPRAGE) and SPM8 on MATLAB platform for post processing statistical analysis.

Results: We find a significant difference between sizes of the cortical maps in ALS patients and healthy volunteers ($p = 0,001$, U-test). The volume of cortical motor maps of APB in healthy volunteers was equal to 83 (64, 102) mm^3 for the left hemisphere and 72 (48, 118) mm^3 for the right. In ALS patients, map volume was equal to -37 [(2, 60) mm^3 and 28 (6, 50) mm^3, respectively. Individual maps obtained for ALS patients were located in the anterior central gyrus, posterior central gyrus and the premotor cortex region. In several ALS patients (with recent ALS onset or relatively benign course), we observed broadening of individual cortical representations: motor evoked potentials were registered in response to stimulation of the frontal and temporal parasagittal regions. There were significant correlations between the

ALSFRS-R and the strength of the contralateral APB with the map size in both right and left hemispheres. The inverse correlation between the map size and the motor threshold was also significant ($p < 0.001$). The area maps are not dependent on disease duration and site of onset. There was a significant correlation between the map size and precentral gyrus VBM volume in left but not in right hemisphere ($p = 0.02$ and $p = 0.3$, respectively).

Conclusions: nTMS and VBM are methods which both describe the condition of cortical motor neurons. The combination of these two techniques in future research could help in the study of neurodegeneration pathogenesis, diagnosis and prognosis.

P88 NEAR-INFRARED SPECTROSCOPY-BASED RESTING STATE ANALYSIS REVEALS ALTERED FUNCTIONAL CONNECTIVITY IN ALS-PATIENTS WITH AND WITHOUT COGNITIVE IMPAIRMENT

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Keywords: cognition, resting state fMRI, near infrared spectroscopy

Background: Extramotor involvement, especially evidenced by executive dysfunction, has been demonstrated in ALS-patients repeatedly (1,2). fMRI-based resting state analyses revealed altered activation in functional networks for ALS-patients, specifically in sensorimotor and default mode networks (3). The latter have been assumed to be associated with cognitive impairment, but have not yet been investigated using near-infrared spectroscopy (NIRS) during the resting state.

Objectives: This study aims to investigate alterations in functional connectivity (FC) in ALS via resting state NIRS-based upon patients' cognitive performance.

Methods: Using a NIRS system consisting of 16 measurement channels, positioned over bilateral prefrontal, temporal and occipital regions, resting state NIRS data were obtained, allowing measurement of homologous and fronto-posterior FC. During measurement participants lay down for 15–20 minutes, keeping their eyes closed in a darkened room. This was done for 33 ALS-patients and 30 age-matched healthy controls (HC). Patients performed a neuropsychological battery of tests comprising Auditory Verbal Learning Test, Complex Figure Test, Digit Span, Trail Making Test, Category fluency and Phonemic verbal fluency, and Stroop Test. They were assigned to groups of pure ALS, executive impaired ALS (ALS-Ex) and non-executive impaired ALS (ALS-NECI) according to the classification suggested by Phukan et al. (2). Data were analysed applying random permutation statistics to identify channel pairs revealing modulations in averaged coherence values in the low frequency (0.06–0.08 Hz) and very low frequency (0.009–0.02 Hz) bands.

Results: Homologous and fronto-posterior connectivity were reduced in ALS-patients compared to HC, as indicated by reduced averaged coherence values for low frequency bands.

Comparisons among ALS subgroups revealed that ALS-Ex patients showed generally increased homologous and fronto-posterior connectivity compared to ALS patients without executive impairment (ALS-NECI). Non-executive cognitive impaired ALS patients showed reduced FC between right and left motor cortex.

Discussion and conclusion: These results suggest that ALS is associated with an alteration of functional brain networks, showing patterns of both decreased and increased FC. Those effects are in line with previous studies that used task fMRI (4) or resting state fMRI (3, 5). Increased FC in the ALS Ex group could be an indicator for neuronal compensation or decreased intracortical inhibition, a phenomenon observed previously in sensorimotor areas in ALS. Decreased connectivity between motor cortices in ALS-NECI could be related with patient motor disability but has to be proven further.

Therefore, we consider that resting state NIRS could be used as an economic and sensitive tool to reflect functional changes of cognitive impairment.

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P89   FRACTIONAL ANISOTROPY LOSS IN ALS EVOLVES AS A FUNCTION OF DISEASE SEVERITY BUT LIMB- AND BULBAR-ONSET DIFFER QUANTITATIVELY WHEN MATCHED FOR SEVERITY STAGE

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Keywords: DTI, fractional anisotropy, imaging

Background: Diffusion tensor MRI studies in Amyotrophic Lateral Sclerosis (ALS) have become popular but the method's true biological worth remains unclear. Studies are somewhat confounded by small, often heterogeneous, groups and inconsistent methodology.

Objective: To understand the evolution of fractional anisotropy (FA) changes in a substantial ALS cohort using a standardized methodology.

Method: N = 45 cases with 'classic' ALS participated in this study; those with dementia, flail limb, or PLS were excluded to maximize clinical homogeneity. Patients and age-matched controls (n = 29) were scanned on a 3T scanner; diffusion data were analyzed using Tract-Based Spatial Statistics focusing exclusively on FA. First, cases were stratified into three sub-groups according to the ALS functional rating scale (1) (ALSFRS-R; mild/moderate/severe: n = 16/15/14; range = 46–43/42-36/35-22) to test whether FA reliably tracks clinical severity. The results led to a hypothesis that bulbar-onset (ALS-b) behaves differently in terms of FA perturbation and ALS-FRS-R score compared to limb-onset (ALS-l). This was tested by stratifying only the ALS-l cases into mild and advanced groups (n = 13/group) and by contrasting ALS-b and ALS-l groups that were strictly matched for power (n = 14/group) and ALSFRS-R score (mean/range): ALS-b = 39.9/29–46, ALS-l = 39.2/27–46, p = 0.6.

Results: The initial stratification by ALSFRS-R score found that the most severe sub-group had the most severe FA reduction (involving corticospinal tracts and body of corpus callosum). The mild and moderate sub-groups showed less severe FA reduction with FA loss worse in the mild compared to the moderate group. Analyzing those with ALS-l only showed clear progression of FA loss from mild to severe. Matched for ALSFRS-R and power, ALS-b showed far worse FA reduction compared to ALS-l even though the ALS-b group had shorter symptom duration (mean months/range = 18/4-50 versus 31/7-81). There were no qualitative differences in lesion distribution for any analyses, only lesion severity varied.

Discussion: The most advanced sub-group in the first analysis had the greatest FA reductions, but paradoxically, FA reductions were worse in the mild compared to the moderate sub-group. Although this could have potentially indicated that the ALSFRS-R lacked construct-validity or that FA changes follow a non-linear evolution, the data suggested a more plausible, third possibility in which an over-representation of ALS-b drove the effect in the mild stratum. This hypothesis was confirmed by comparing ALS-b and precisely matched ALS-l sub-groups. Stratifying ALS-l revealed clear progression of FA loss with advancing disease.

Conclusion: FA has strong potential as a severity biomarker in ALS. There is a critical confound, however, regarding ALS-l versus ALS-b that must be considered in cross-sectional studies. ALS-b appears to be a truly more aggressive variant when FA loss and symptom duration are considered but effects the same regions as ALS-l.

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P90   ORIGIN OF FASCICULATIONS IN ALS AND BENIGN FASCICULATION SYNDROME

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Keywords: benign fasciculations, fasciculation potentials, electromyography

Background: The site of origin of the fasciculation potentials (FPs) in ALS and other disorders has proven difficult to determine. Most evidence favors distal origin, but central origin cannot be discarded. The origin of benign FPs (BFPs) has not been investigated.

Objectives: We aimed to study the origin of FPs in ALS and in subjects with benign FPs with a novel approach.

Methods: We studied 52 ALS patients, mean age 59.6 ± 10.1 years, means disease duration 11.1 ± 5.7 months, referred for diagnostic testing. All had one tibialis anterior (TA) of normal strength. We studied 11 patients with BFPs, mean age 58.5 ± 11.7 years. These patients had normal motor unit potential (MUP) analysis and no progression to other disorders. We classified the TA muscles in the ALS patients as with or without neurogenic change, according to MUP analysis. Recordings were made from two adjacent sites in the TA muscle using two concentric needle electrodes placed at least 1cm apart perpendicular to the vertical axis of the muscle. The peroneal nerve was stimulated at low intensity at the fibular head to confirm that the two electrodes were recording from MUs innervated by different axons. Between 6 and 8 pairs of recording sites were used in each subject. We quantified the number of FPs recorded in time-locked fashion at both of the two sites in each recording.

Results: Thirty-two ALS patients showed neurogenic change in the TA muscle. In these patients, 1096 FPs were recorded; 941 (85.7%) of these were recorded from only one of the recording site – the remaining 155 (14.3%) were recorded time-locked. Twenty ALS patients had TA muscles with normal MUPs, 544 FPs were recorded, 394 (72.7%) were recorded from only one site and 150 (27.3%) were recorded time-locked from both sites. In the 11 subjects with BFPs, 234 FPs were recorded; 129 (55.1%) FPs from only one electrode; 105 (44.9%) from the two sites. The probability of recording time-locked FPs was greater in the BFPs group than in the two groups of ALS patients ($p < 0.0001$, chi-square test). The probability of time-locked FPs in the two recording sites was significantly greater in the ALS group without neurogenic changes than in ALS with neurogenic changes ($p < 0.0001$, chi-square test).

Discussion and conclusion: Our results show that time-locked FPs recorded from two MUs innervated by different axons in a muscle, are more common in benign fasciculation than in ALS. In addition, in ALS, time-locked FPs were more common in TA muscles not yet involved by denervation and reinnervation than in muscles in which there was chronic partial denervation. We conclude that the origin of FPs changes over disease progression in ALS.

P91   SYMPTOM SPREAD IN PRIMARY LATERAL SCLEROSIS IS CONSISTENT WITH PATHOLOGY THAT SPREADS THROUGH AXONAL PATHWAYS AND TO CONTIGUOUS BRAIN REGIONS

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Keywords: primary lateral sclerosis, symptom progression, chart review

Background: Two opposing theories have been proposed to explain how disease spreads in motor neuron disease as symptoms progress from one body region to another. One proposes that degeneration occurs in CNS areas with axonal connections, while the other proposes a prion-like spreading of misfolded proteins. The sequence of symptom progression in primary lateral sclerosis, which selectively affects upper motor neurons, can test these proposals. Local spreading of misfolded proteins should produce symptoms in contiguous somatotopic areas of the motor strip, whereas spread through network connections would involve spread to non-contiguous areas, for example, via the corpus callosum.

Objectives: To determine whether the spread of clinical symptoms is consistent with spread to contiguous areas or through axonal connections in PLS.

Methods: Charts of 45 patients followed at NIH for a clinical diagnosis of PLS were reviewed. Each instance of symptom onset in an additional body region was categorized as consistent with contiguous spread, through axonal connections, possibly contiguous, or unrelated/undetermined.

Results: In the 45 patients, there were 152 instances where symptoms progressed to additional body regions. One hundred and sixteen instances could be classified as axonal or contiguous spread according to a set of classification rules. Seventy-seven instances were consistent with contiguous spread, and 39 were consistent with axonal spread. The first instance of symptom spread was approximately equally divided between instances classified as contiguous or axonal. Symptoms began in a lower extremity in 37 patients; in 22 of these patients, the next symptoms involved the other leg, which we classified as axonal spread. However, in some patients the arm ipsilateral to the first leg became symptomatic about the same time as the contralateral leg, and that spread was classified as contiguous. Following the initial instance, subsequent instances of symptom progression were predominantly classified as contiguous. There was no difference between the average time for instances of axonal and contiguous spread (2.17 ± 1.8 vs. 2.42 ± 1.77 years, respectively).

Discussion: Symptom spread in PLS is consistent with a mixture of pathology that spreads through axonal pathways and contiguous brain regions. The predominance of axonal spread early in the disease course is partly due to the common occurrence of symptoms in one leg followed by the other. Later in the course of the disease, instances classified as contiguous spread were more prevalent, although the average interval for symptom spread was the same for axonal and contiguous instances.

Conclusions: In PLS, symptom spread from one body region to another is consistent with a mixture of spread through axonal pathways, especially early in the disease, and local spread to adjacent cortical regions, particularly later in the course of the disease.

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P92 ALTERED GLOBAL AND LOCAL RESTING STATE BEHAVIOUR IN AMYOTROPHIC LATERAL SCLEROSIS AT BASELINE AND DISEASE PROGRESSION

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Keywords: MRI, resting state fMRI, disease progression

Background: ALS is primarily characterized by degeneration of neurons in the motor cortex (M1). Task motor fMRI studies suggested a cortical reorganization, where activity of sensorimotor network regions (4) was enhanced but decayed with increasing weakness (3). So far, there is no investigation on resting state behaviour in the course of the disease.

Objectives: This study aimed to investigate functional connectivity (FC) and spontaneous fluctuations via resting state MRI measures in the progression of the disease.

Methods: Eighty-one ALS-Patients and 68 controls (HC) underwent a resting state scan (3T). FC for regions of interest of the left and right M1 and fractional amplitude of low-frequency fluctuation (fALFF) (5) were calculated voxelwise for patients and HC, and correlated with ALSFRS-R scores in patients. After 3–6 months, 42 patients were measured again and maps for FC and fALFF were compared to baseline. Significant clusters were corrected for multiple comparisons ($p < 0.05$).

Results: ALS patients showed significantly higher FC seeded from right M1 with the ipsilateral and contralateral precentral gyrus and supplemental motor area (SMA) in comparison to HC. Decreased FC from right M1 with the default mode network was observed for ALS patients compared to HC. FC of both M1 towards contralateral precentral gyri correlated positively with patients' disease severity as well as fALFF in left and right premotor cortex. There was an inverse correlation between patient ALSFRS-R score and fALFF in the cerebellum. Longitudinal decreases of FC of M1 were found towards ipsilateral SMA and contralateral precentral gyrus while fALFF decreased in the left precentral gyrus at follow-up.

Discussion and conclusion: We demonstrate that alterations of motor FC in ALS coincide with altered local fluctuation amplitudes in M1 and the cerebellum in dependence of the clinical severity. Such network alterations are in accordance to previous studies that used resting state fMRI (1,2), considering therefore rsfMRI as a useful biomarker for disease severity and progression.

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P93 DISTAL ULNAR AND MEDIAN NERVE APPEARANCE IN ALS PATIENTS WITH DIFFERENT PHENOTYPES – A PROSPECTIVE ULTRASOUND STUDY

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Keywords: high-resolution ultrasound, PLS, ulnar nerve

Background: Amyotrophic lateral sclerosis (ALS) is characterized by a progressive axonal degeneration of the upper and lower motor neurons. In primary axonal neuropathies high-resolution ultrasound revealed increased cross-sectional areas (CSA) and fascicle diameters (FD) of peripheral nerves. Indeed, considering ALS and its clinical phenotypes, the sonographic nerve appearance and the diagnostic value of high-resolution ultrasound have not been determined so far.

Methods: CSA and FD of the median and ulnar nerves at the forearm and wrist were investigated sonographically (12-MHz-probe) in 68 patients with ALS (bulbar phenotype n = 15, classic subtype n = 19, lower motor neuron disease variant (LMND) n = 19, upper motor neuron disease variant (UMND) n = 15), in six patients with primary lateral sclerosis (PLS) and in 18 matched healthy controls. All patients obtained electrophysiological measurements of the median and ulnar nerves.

Results: Patients and controls with carpal and/or cubital tunnel syndromes were excluded from statistical analyses. Compared to the healthy volunteers all ALS patients exhibited significant reductions of the ulnar nerve CSA at their forearm and wrist which was applying to all investigated phenotypes. Contrary, in the PLS group the ulnar nerve CSA did not differ from those values detected in the controls. There were no differences of the median nerve CSA as well as of the median and ulnar nerves' FD between the ALS phenotypes, PLS and control groups. Preliminary analyses revealed an inverse association between age, duration of illness and ulnar CSA in ALS. Concomitantly, corresponding to their ulnar pathology detected by high-resolution ultrasound, about 40% of all ALS patients exhibited increased ulnar distal motor latencies and in about 20% reduced motor action potential amplitudes were detected.

Conclusions: All ALS phenotypes apart from PLS exhibit a relatively uniform sonographical appearance predominantly of the distal ulnar nerve potentially facilitating an easier clinical discrimination between, for example, the UMN-dominant variant of ALS and PLS, a spastic syndrome of slow progression.

P94   A RETROSPECTIVE STUDY OF ELECTROMYOGRAPHY OF THE TRAPEZIUS MUSCLE AT TIME OF DIAGNOSIS AS A PREDICTOR OF EARLY RESPIRATORY IMPAIRMENT IN ALS PATIENTS

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Background: In ALS, death is usually secondary to respiratory failure (1, 2). Recent studies have shown that decreased motor responses of the phrenic nerve are important prognostic factors for respiratory failure (3) but phrenic testing is not routinely performed and is technically difficult. Tests which are easier to administer and more tolerable to patients may be more useful in at the early identification of those at risk of early respiratory failure (4).

Objective: To examine for an association between the presence of active denervation on electromyography (EMG) of the trapezius muscle and reduced respiratory function in newly diagnosed patients with ALS.

Methods: This study is a retrospective chart review. The charts of all deceased patients the Calgary ALS and Motor Neuron Disease Clinic from 2006–2010 were considered for this study.

Patients who had been diagnosed as having clinically definite; clinically probable; clinically probable (laboratory-supported); clinically possible; clinically suspected ALS according to the El-Escorial diagnostic criteria for ALS were eligible for the study. Patients having both EMG studies of the trapezius and forced vital capacity (FVC) testing upon presentation were included. The proportion of patients with trapezius denervation who had reduced FVC (less than 90% of predicted) was calculated. FVC at the last clinic visit was also recorded, allowing the calculation of rate of change in FVC over during the period of active disease.

Results: We reviewed 197 deceased ALS patient charts and identified 42 reporting electromyography of the trapezius muscle. Signs of denervation were observed in 19/42 (45.2%) of as evidenced by the presence of fibrillations or positive sharp waves. Reduced FVC was observed in 28/42 (66.7%) at presentation. Patients with active denervation of the trapezius muscle at time of presentation were not more likely to demonstrate reduced FVC at presentation but had a statistically greater rate of FVC decline then those without denervation (p = 0.0078).

Discussion and conclusion: There is evidence of denervation in the trapezius muscle upon first presentation with ALS in a significant proportion of the patients. The correlation between trapezius denervation and increased rate of FVC decline may prove to be a good predictor of future respiratory compromise in ALS.

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P95   DECREASED PLASMA LEVELS OF FIBRONECTIN IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Fibronectin (FN) is a large glycoprotein (440 kDa) present in many tissues and in most body fluids. Important properties of FN include its ability to bind to the cell surface and the fact that it has other binding sites to recognize the collagen molecules and other connective components. In this way, FN is believed to be involved in the interaction of cells with the extracellular matrix, and thus influence both morphologic properties and differentiation. Recent investigations have shown that increased levels of serum type III procollagen and serum hyaluronic acid, and decreased levels of serum 7S collagen are associated with amyotrophic lateral sclerosis (ALS). All of these factors are known to be involved in the metabolism of connective tissues. However, there have been no published studies of plasma FN in ALS.

Objectives: To study plasma levels of FN in ALS.

Methods: Our subjects were 28 patients with ALS, 18 control subjects with other neurological diseases (control group A) and 21 healthy adults (control group B). Plasma FN concentration was determined by turbidimetric immunoassay. FN was reacted with monospecific antibody in aqueous solution. The immunocomplexes which were formed caused some turbidity. Turbidity was found to be linearly dependent on FN concentration up to an absorbance reading of 200/cm at 340 nm.

Results: In ALS patients and control groups A and B serum levels of FN were 186.6 ± 93.3 µg/dl, 271.7 ± 122.6 µg/dl, and 325.7 ± 114.4 µg/dl, respectively. Serum FN levels were significantly lower ($p < 0.02$ and $p < 0.001$, respectively) in ALS patients than in control groups A and B. There was no marked difference ($0.1 < p < 0.5$) in serum levels of FN between control groups A and B. There was a significant negative correlation ($r = -0.70$, $p < 0.001$) between serum FN levels and duration of illness in ALS patients, but there was no such correlation in control group A. No relationship was found between concentrations of serum FN and dysphasia, muscle power, and severity of disability in ALS patients or control group A.

Discussion and conclusion: The low values of plasma FN in ALS patients may be accounted for by increased catabolism of FN mediated by the presence of altered homeostasis. The postulated causes of the decreased plasma FN levels include altered turnover, increased consumption, altered tissue distribution, and decreased production of this substance, and there is as yet no consensus on this subject. As indicated above, decreased plasma FN levels may be related to the pathogenesis of ALS. The measurement of plasma FN may be useful as a supplementary and noninvasive test in the diagnosis of ALS.

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P96   PLASMA NEUROFILAMENTS AS A BIOMARKER OF DISEASE PROGRESSION IN ALS: INSIGHTS FROM LONGITUDINAL STUDIES IN MICE AND MAN

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Keywords: neurofilament heavy chain, biomarker, disease progression

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder for which there is no effective treatment. Clinical trials in ALS are hindered by the lack of reliable biomarkers to enable an early diagnosis and monitoring of disease progression. There is therefore an urgent need to develop biomarkers for ALS.

Objectives: To determine whether plasma neurofilament heavy chain (NfH) levels are a biomarker of disease progression in mouse models and ALS patients.

Methods: Blood was drawn from SOD1^{G93A} mice at various disease stages, when neuromuscular function and motor neuron survival were also assessed. ALS patients ($n = 136$) were evaluated in clinic and blood samples taken every 3 months. Disease progression was evaluated using the ALS Functional Rating Scale_Revised (ALSFRS_R). Controls include healthy controls and neurological disease controls ($n = 104$). Plasma NfH levels were determined using an in-house ELISA method. A cross-sectional study of NfH levels at baseline sampling as well as a longitudinal study of serial samples from ALS patients was undertaken.

Results: In SOD1^{G93A} mice, we observed a significant increase in plasma NfH levels as disease progressed, which correlated with the decrease in functional and morphological read-outs of disease. In contrast, in a cross-sectional study of 136 clinically heterogeneous ALS patients and 104 controls, we found comparable levels of plasma NfH levels. Longitudinal analysis of plasma NfH levels showed that patients with a shorter diagnostic latency and disease duration progressed faster and had higher plasma NfH levels during the early stages of the disease which declined later on

with disease progression. Paradoxically, low levels of plasma NfH correlated with a poor prognosis. Importantly, in our large heterogeneous ALS cohort, we found that changes in plasma NfH levels did not predict disease progression. Contrary to expectations, plasma NfH levels do not always increase as disease progresses in ALS patients, possibly as a result of NfH aggregate formation and cleavage. Initial analyses reveal the presence of auto-antibodies to NfH in ALS patient plasma, suggesting that the immune response may influence measurement of plasma NfH.

Discussion: Our results shown that plasma NfH levels are a good biomarker of disease progression in SOD1^{G93A} mice, which exhibit a well characterised, aggressive disease phenotype. However, in clinically heterogeneous ALS patients, changes in plasma NfH levels during disease progression are more complex. Our data suggest that the asymptomatic phase of ALS and the delay to diagnosis or to baseline sampling have a significant impact on NfH measurements performed in the symptomatic phase of the disease of ALS individuals.

Conclusions: The diagnostic delay from disease onset to diagnosis has a significant and confounding impact on the analysis of plasma-based biomarkers for ALS, including NfH as well as other pathologically relevant neurochemical markers in ALS.

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P97   EOSINOPHIL-DERIVED NEUROTOXIN AS A BIOMARKER FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: eosinophil-derived neurotoxin, neuroinflammation, biomarker

Background: Oxidative stress and neuroinflammation have been implicated in the pathophysiology of amyotrophic lateral sclerosis (ALS). There are prevalent danger signals involved in ALS. Many inflammatory molecules including interleukin-6, interferon-γ, tumor necrosis factor-α, and nitric oxide have been reported to be elevated in the serum of ALS patients. Members of damage-associated molecular patterns, including reactive oxygen species, eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein (ECP) may also participate in its pathogenic processes. Herein, we hypothesized that two important danger signals, EDN and ECP, may elevate in ALS patients and be used as biomarkers for ALS.

Methods: Forty-four ALS patients, 39 patients with Alzheimer's disease (AD), 40 patients with Parkinson's disease (PD), and 44 age-matched healthy controls were recruited from Taipei City Hospital – Zhongxiao Branch. Detailed clinical data of these patients were registered. 10ml venous blood was collected from each participant. The concentration of serum ECP and EDN was measured using an enzyme-linked immunosorbent assay. ANOVA, Bonferroni multiple

comparison test, and Spearman rank correlation were used for statistical analysis.

Results: The average EDN levels of ALS, AD, PD patients, and healty controls were 24.1 ± 24.5 ng/ml, 15.4 ± 17.4 ng/ml, 15.8 ± 15.2 ng/ml, and 21.1 ± 27.4 ng/ml, respectively. Averaged EDN level of ALS patients was significantly increased 2.17 fold as compared with that of healthy controls ($p < 0.05$). There was no significant correlation between the levels EDN and various clinical parameters of ALS patients. The serum EDN level of ALS patients was significantly increased 1.61 and 1.84 fold as compared with that of AD and PD patients, respectively. There was no significant difference for ECP levels between patients with ALS, AD, PD and healthy controls. Meanwhile, there was no significant correlation between ECP levels and each clinical parameter. Hence, EDN, but not ECP, may serve as an indicator for ALS. Receiver operating characteristic analysis of EDN for diagnosis of ALS showed best performance with 88.53% accuracy, 77.27% sensitivity, 84.09% specificity when cut-off value was set at 23.43 ng/ml. The value of area under the curve was 0.8264.

Conclusion: Our study showed EDN levels were significantly elevated in ALS patients. We propose that EDN may participate in the pathogenesis of ALS and may serve as a biomarker for ALS.

P98   SERUM FERRITIN IS ELEVATED IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: serum ferritin, iron metabolism, biomarker

Background: Dysregulated iron metabolism may play a role in ALS pathophysiology. Polymorphisms in the *HFE* iron regulatory gene have been shown to increase ALS risk, and iron dyshomeostasis negatively impacts pathways implicated in ALS, including oxidative stress.

Objectives: To analyze levels of serum ferritin, a marker of iron metabolism, in ALS patients as well as healthy and diseased controls, and to determine if ferritin levels impact survival.

Methods: An IRB-approved, retrospective analysis was performed of data from 137 ALS patients, 152 healthy controls, and 80 patients with other neurological diseases seen at a university-based multidisciplinary ALS clinic or who provided samples to the Northeast ALS Consortium. Gender, age, site of onset, and, for selected patients, date of symptom onset as well as death was recorded. Survival time was defined as the duration from symptom onset to death. Serum ferritin levels were measured using standard clinical laboratory procedures. One-way ANOVA was used to compare serum ferritin levels between groups, whereas Spearman correlation was used to measure the association between serum levels and survival time. Serum ferritin levels were categorized into high and low groups, and survival analysis was performed using Kaplan–Meier logrank statistics and Cox proportional hazards regression.

Results: Age was significantly different in ALS patients (mean 60.5 years) compared to that of healthy (mean: 44.1) and diseased controls (mean: 52.1). However, age was not associated with serum ferritin levels (R-squared 0.063, canonical correlation). Gender proportions were significantly different in ALS patients (65.3% males) compared to that of healthy (32.5% males) and diseased controls (38.5% males), and gender significantly affected serum ferritin levels (Male: mean $= 224.8$ ng/ml and Female: mean $= 90.0$ ng/ml $p < 0.001$). Therefore, serum ferritin comparisons between groups were stratified for gender. In males, serum ferritin levels were significantly higher in ALS patients (mean: 286.6 ng/ml) than those of either healthy (mean: 160.8 ng/ml, $p < 0.001$) or diseased controls (mean: 164.5, $p = 0.003$). In females, serum ferritin levels were significantly higher in ALS patients (mean: 142.6 ng/ml) than those of either healthy (mean: 69.3 ng/ml, $p < 0.001$) or diseased controls (mean: 77.5, $p < 0.001$). However, serum ferritin levels were not associated with survival times in either males (R^2: 0.001) or females (R^2: 0.069). ALS patients were categorized into low and high ferritin groups by median values (200.0 ng/ml, males; 123.0 ng/ml, females). Ferritin status did not significantly impact survival by Kaplan–Meier logrank tests or Cox regression in either gender.

Discussion and conclusion: These results suggest altered iron metabolism in ALS patients, effects that are not simply due to presence of neurological disease. However, serum ferritin alone may be insufficient to reflect or predict disease progression and survival.

P99   URINARY EXTRACELLULAR DOMAIN OF NEUROTROPHIN RECEPTOR P75 MEASUREMENTS AS A NOVEL BIOMARKER FOR MOTOR NEURON DISEASE

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Keywords: biomarkers, human urine, ELISA

Background: Objective biomarkers for motor neuron disease (MND) would aid diagnosis and facilitate the discovery of new treatments. Sources of biomarkers such as urine that are easily obtained should be investigated, as the invasive nature of CSF sampling is problematic for MND patients and especially healthy controls (1). p75NTR re-expression after nerve injury and cleavage of the extracellular domain of p75NTR (p75NTRECD) is part of a homeostatic program that removes defective neurons, axons and synapses upon injury and degeneration (2). In MND patients, p75NTR is up-regulated in motor neurons and Schwann cells post-mortem, but not in healthy controls (3, 4). We have previously found p75NTRECD in urine of MND mice and patients (5). We now ask whether levels of urinary p75NTRECD serve as a useful diagnostic and progression biomarker for MND.

Methods: A quantitative sandwich ELISA was used to detect p75NTRECD in the pg/ml range. Mass spectrometry

and western blotting (WB) of urinary p75NTRECD after immunoprecipitation (IP) confirmed p75NTRECD in MND patient urine. Urine and neurological data were collected from 26 patients with sporadic MND, 12 healthy controls and 19 patients with non-MND diseases including Parkinson's disease (PD) and Multiple Sclerosis (MS). p75NTRECD was also measured multiple times from a cohort of 14 MND patients.

Results: The presence of p75NTRECD in urine of MND patients was confirmed by IP/WB and mass spectrometry. Urinary p75NTRECD levels measured by ELISA predict MND from healthy controls with high levels of sensitivity and specificity. The mean value for urinary extracellular p75NTRECD from 26 MND patients was 7.45 ± 0.53 (ng p75NTR/mg creatinine) significantly higher ($p < 0.001$) than 12 healthy control patients (2.5 ± 0.22) and 19 patients with other neurological diseases (4.7 ± 0.31; PD and MS). Urinary p75NTRECD tested over 3-month intervals in 14 MND patients is being performed with preliminary data indicating that p75NTRECD levels increase with a decline in the amyotrophic lateral sclerosis functional rating scale-revised (ALSFRSr). In addition, high urinary p75NTRECD appears to be prognostic for decreased survival time ($n = 9$).

Discussion and conclusion: These findings indicate urinary p75NTRECD offers utility as a biomarker MND. In addition, preliminary results indicate that it may be useful as a progression and prognostic marker. p75NTRECD should now be evaluated in large population studies.

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P100   IRISIN, A NEWLY IDENTIFIED MYOKINE AS A CANDIDATE MARKER OF METABOLIC INVOLVEMENT IN ALS

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Keywords: irisin, biomarker, pgc-1a

Background: The development and ascertainment of biomarkers of disease onset and progression is a key objective of clinical ALS research and an important prerequisite for therapeutic trials. In addition to this identification of biomarkers might offer insights in to disease pathogenesis. Transcriptional processes regulated by peroxisome prolifer-ator-activated receptor gamma (PPARg) coactivator-1 alpha (PGC-1a), which are critical for metabolic regulation and mitochondrial biogenesis, have been shown to be impaired in ALS and other neurodegenerative diseases. Therefore monitoring function of the PGC-1a system is of great potential interest in ALS. A protein which is under the direct transcriptional control of PGC-1a is the newly identified hormone called Irisin. Irisin is 112 amino acids long and is a cleavage product of the precursor protein FNDC5. It exists in at least four isoforms. It was originally described in 2012 as a myokine that signals metabolic infor-mation from the muscle to the adipose tissue and mediates many of the beneficial metabolic effects of exercise, for example, improved glucose tolerance. Irisin has thus been dubbed the 'exercise hormone' and it can be detected in blood serum.

Methods: Western blot analysis and ELISA were used to measure Irisin blood levels. An in-house ELISA for detec-tion and quantification of Irisin in serum was developed in the Department of Internal Medicine I, Ulm University. Patient groups consisted of patients with motor neuron disease (MND) ($n = 10$; 41.6–74.6 years); patients with Huntington's disease, HD ($n = 5$; 39.7–54.3 years), and age-matched normal controls without any neurological disease.

Results: Irisin serum levels were found to be reduced in HD and ALS compared to those of controls. Irisin is a candidate marker of metabolic pathway involvement in ALS and HD. The metabolic dysregulation is of potential relevance for understanding of the disease pathogenesis.

Discussion: Further studies are needed to study the regula-tion of Irisin levels and to elucidate the mechanisms underly-ing these effects.

P101   AN IMPROVED MOTOR UNIT NUMBER INDEX (MUNIX) AS BIOMARKER FOR ALS

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Keywords: motor unit number estimation, motor unit number index, neurodegeneration

Objective: Biomarker improvement and validation of the new non-invasive neurophysiological method MUNIX (motor unit number index). The reliability, practicability and inter-rater-variability of potential improvements to MUNIX were determined and compared to two established Motor Unit Number Estimation (MUNE) methods.

Methods: Forty healthy subjects and 18 patients with amyo-trophic lateral sclerosis (ALS) were studied prospectively at single point or multiple points in time. MUNIX results were compared with incremental stimulation MUNE (IS-MUNE) at abductor digiti minimi muscles (ADM), and with spike-triggered averaging MUNE (STA-MUNE) at trapezius mus-cles (TRA). In contrast to the original MUNIX method, we recorded a continuous electromyogram during increasing muscle contraction to reduce the influence of both patient's compliance and investigator bias. Moreover, baseline correc-tion for CMAP was implemented and the influence of the

parameter settings (filters, number of data points, and rectifying) was systematically studied.

Results: The best parameter setting includes high pass-filter 10Hz, low pass-filter 3000Hz, number of data points 1000, and rectification of the signals. This leads to an improved correlation between MUNE and MUNIX, up to r = 0.80 for ADM and r = 0.7 for TRA. The inter-rater-variability is expected to be below 10% and therefore considerable better than for IS-MUNE/STA-MUNE.

Conclusion: The improved MUNIX needs minimum patient cooperation, and provides stable results easily and quickly. Improved MUNIX is suggested as an excellent biomarker alternative to the established MUNE methods in distal and as well as in proximal muscles.

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P102 "REPORTING BIOMARKER" DEVELOPMENT: UPDATE IN ALS PATIENTS TREATED WITH G-CSF-MOBILIZED HEMATOPOIETIC STEM CELLS

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Objective: Treatment development in neurodegeneration is demanding because of time slots for evaluating effects and multiple confounders. We selected prolonged open label 'compassionate use' autologous BM stem cell mobilization in human ALS-patients to pre-validate 'reporting biomarkers' for disease modulation and safety evaluation.

Methods: Twenty ALS patients were treated with s.c. rec-hu-G-CSF, in a conventional 5/28 days or a 1/7 days × 4 (each = 1 cycle) outpatient regimen: 5–10 μg/kg BW were given daily plus riluzole. Patients with a median age of 48 yrs (25–75y, 13m and 7f,) were evaluated by ALS-FRS-R every 4 weeks. As reporting biomarkers we assessed (1) hypothenar muscle motor unit number estimates (MUNE, Mc Comas) and (2) cranial MRI-DTI to delineate microstructural FAI-changes as sign of axonal damage/repair in motor cortex and pyramidal tracts every 12 weeks, and (3) BM function including stem cell differentiation profile, smears and blood counts, Burst forming and colony forming units (BFU-E, CFU-E, CFU-GM, CFU-GEMM) pre- and post-mobilization. Safety included abdominal sonography, determination of BW, pulmonary function, and clinical chemistry.

Results: Both application modes (5/28: 15 treatment cycles (4–31) and 1/7x4: 11.5 cycles (4–16)) were safe with no obvious difference in efficacy. Side effects were very mild and tolerance was good. No differences were detected when compared to an ALS population treated with riluzole only. Clinical outcome revealed some longer stabilisations, unrelated to age or disease dynamics. Median overall survival in application mode 5/28 was 2.9 years. MUNE correlated to disease progression (p < 0.01) and DTI-FAI (p < 0.003), and showed increases in individual patients. DTI-/FAI-values over time indicated decrease in most patients, in some minor improvement. BM function was complex, and correlations to effects are still ongoing. Median overall survival could not yet be determined.

Discussion: Prolonged treatment with G-CSF is feasible and safe in ALS patients; however, prospective study data are needed. MUNE, DTI and BM function parameters are probably very useful biomarkers.

P103 THE FEATURES OF SERUM LIPID AND SURVIVAL IN ALS PATIENTS: A STUDY FROM SOUTHWEST CHINA

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Keywords: amyotrophic lateral sclerosis, serum lipid, survival time

Background: To investigate the associations between fasting serum lipid and survival in Chinese ALS patients.

Methods: Four hundred and thirteen ALS patients (241 males and 172 females) were included in the study. All the patients were regularly followed up from 2004 to 2013. Fasting serum lipid concentration of all the subjects, consisted of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were measured at the time of first visit. Information including survival time, and revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALS-FRS) were collected by follow-up.

Results: ALS patients with high triglyceride serum levels had longer survival time compared with ALS patients with lower triglyceride serum levels (p < 0.05). We found a median prolonged life expectancy by 5.66 months for patients with serum triglyceride levels above the median of 1.47mmol/L. A meta-analysis indicated that there were no significant differences in mean total cholesterol, triglycerides, LDL and the LDL/HDL ratio between ALS patients and controls.

Conclusion: High triglyceride serum levels may prolong survival time and maybe a potential prognostic factor in ALS patients.

P104 VIDEOFLUOROGRAPHIC MARKERS IN SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA): A STUDY OF 111 JAPANESE PATIENTS

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Keywords: SBMA, videofluorography, dysphagia

Objective: SBMA is a lower motor neuron disease characterized by weakness and atrophy of limb, facial, and oropharyngeal muscles. Dysphagia is a major symptom of SBMA and often leads to life-threatening events such as aspiration pneumonia and suffocation. Although bulbar dysfunction is an important prognostic factor in SBMA, pathophysiology of dysphagia remains elusive. The purpose of this study is to clarify the characteristics of dysphagia in spinal and bulbar muscular atrophy (SBMA) using videofluorography (VFG) and investigate the relationship between bulbar palsy and other clinical phenotypes of the disease.

Methods: We performed VFG examination on 111 consecutive genetically confirmed SBMA patients and analyzed quantitative and qualitative data. In each VFG study, swallowing of 3 ml liquid barium was viewed in the lateral plane and was analyzed using Logemann's videofluorographic examination of swallowing worksheet. The VFG findings were compared with 53 healthy age-matched controls using t-test and chi-square test. Quantitative VFG data were also compared with patient genetic background such as CAG repeat length, and with other motor functional measures such as ALSFRS-R using Pearson's correlation coefficient.

Results: All the patients examined demonstrated qualitative abnormalities in VFG. Most pertinent abnormal findings include nasal penetration, vallecular residue after swallow and insufficient tongue movement ($p < 0.05$ for each). Quantitative analyses showed that ALSFRS-R bulbar components correlated with oral residue and piecemeal deglutition.

Conclusion: Dysphagia in SBMA is characterized by impaired tongue movement in the oral phase followed by vallecular residue and piecemeal deglutition.

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THEME 5 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

THEME 5 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P105   PROGNOSTIC FACTORS FOR SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS IN SERBIAN POPULATIONP

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Background: A variety of prognostic factors have been associated with a shorter survival including older age at onset, female sex, bulbar onset, and short time elapsed from first symptoms to diagnosis. Accurate information about prognosis of ALS is useful to patients, families, and clinicians.

Objectives: The aim of the study was to determine the survival and prognostic factors of ALS patients in Belgrade district (Serbia), over a period of 17 years (1992–2009).

Methods: A retrospective analysis has been carried out on all ALS patients collected by analyzing hospital in- and out-patient registers at the Clinic of Neurology, Clinical Center of Belgrade and in other three clinical centers in Belgrade from January 1992 to December 2009. The diagnosis of probable or definite ALS was based on the El-Escorial revised criteria. Demographic and clinical data, including age at onset, age at diagnosis, the time between onset and diagnosis, the site of onset (spinal vs bulbar), site of onset of motor neuron involvement, treatment (riluzole, PEG, and NIV), and time of death were recorded for all patients. ALS Functional Rating Scale-Revised (ALSFRS-R) was used at the initial evaluation to assess motor function. Each patient was regularly followed up during the disease. Survival time was defined as the time from the onset of symptoms until the death. All statistical analyses of the association between clinical manifestations and the survival pattern were analyzed using the SPSS 17.0 software.

Results: During the analyzed period, 325 ALS patients were diagnosed in Belgrade district. The mean age of the onset for all examined ALS patients was 57.74 ± 11.46 years (range: 25–83 years) and the mean age at diagnosis was 59.21 ± 11.26 years. There were 267 (82.15%) deaths during the study period, 160 (59.92%) men and 107 (40.07%) women. There were 194 (80.49%) patients with spinal onset and 73 (86.90%) patients with bulbar onset. The mean survival was 4.35 ± 0.20 years and the median survival was 3 ± 0.14 years (36 ± 1.68 months). Overall survival from symptom onset was 3 years in 197 (60.6%), 5 years in 68 (20.9%), 10 years in 48 (14.8%) patients and more than 10 years in 10 (3.1%) patients. The cumulative probabilities of survival in a 2-year, 5-year, and 7-year intervals were 71%, 24%, and 17%, respectively. In multivariable Cox proportional hazards regression models, factors which significantly and independently were associated with prognosis included age at onset, diagnostic delay ALFRS score, and use of riluzole.

Conclusion: These findings are consistent with other population-based studies and confirm that older age, shorter diagnostic delay, and ALSFRS score below 39 are strong predicators of poor prognosis of ALS. Treatment with riluzole improves ALS survival.

106   ELEVATED CREATINE KINASE IS ASSOCIATED WITH A BETTER PROGNOSIS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Creatine kinase (CK) is an enzyme found in skeletal muscles, myocardium and brain (CK-MM, CK-MB, and CK-BB isoenzymes, respectively). In these tissues with high energy requirements, CK catalyses the conversion of phosphocreatine to creatine generating adenosine triphosphate. Elevated CK-MM is considered a marker of muscle damage. In the past, CK has been used to differentiate between myopathic and neurogenic disease, higher CK being associated with myopathic disease. It is recognised that CK may be mildly to moderately elevated in patients with amyotrophic lateral sclerosis (ALS). An obvious explanation of raised CK in ALS is striate muscle atrophy resulting from degeneration of the innervating motor neurons. Another possible explanation is that compensatory upregulation of this enzyme may provide an energy substrate in a hypercatabolic condition. The reason that CK elevation occurs in a proportion of ALS cases, the precise cause and its behaviour with disease progression is unknown.

Objectives: To determine (1) whether ALS patients with raised CK differ from patients with normal CK; (2) the implications of raised CK on disease outcome or prognosis; (3) whether CK level mirrors disease progression or activity and, if so, can it be used to monitor disease progression or response to therapeutic interventions?; and (4) Whether the magnitude of muscle enzyme release can be used to predict the magnitude of muscle functional impairment.

Methods: This is an observational cohort study using the clinical database from the Olesoxime (TRO19622) investigational medicinal product trial. This trial involving 512 patients with ALS was conducted across 15 European centres (2009–2011). The patients were followed up at three monthly intervals for 18 months, with monitoring of biochemical and haematological parameters, including CK. The steering committee of TROPHOS (the Biotechnology company developing Olesoxime) kindly provided the database from the Olesoxime trial to conduct this study.

Results: Baseline CK was raised in 52% of the participants; mean CK $257 \pm$ SD of 239 IU/L. Mean CK in male participants was significantly higher than in females ($p < 0.001$). Mean CK was significantly higher amongst participants with limb onset ALS compared to participants with bulbar onset ALS ($p < 0.001$). There was no significant difference in CK levels between upper limb and lower limb onset disease ($p = 0.746$). A higher CK level was associated with significantly better survival, even when adjusted for prognostic covariants ($p = 0.005$).

Conclusions: CK level is a prognostic factor for survival in ALS, independent of its association with site of disease onset. This finding suggests that CK may be involved as a defence mechanism in the face of the metabolic stress of ALS. CK levels cannot be used to predict the disease severity or manual muscle scores at any time point. This study highlights the potential usefulness of CK as a prognostic marker in ALS.

P107   MYOCARDAL INFARCTION AND TAKO-TSUBO CARDIOMYOPATHY IN FEMALE PATIENTS WITH BULBAR AMYOTROPHIC LATERAL SCLEROSIS: A POSSIBLE RELATIONSHIP?

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Keywords: Tako-Tsubo, myocardial infarction, autonomic dysfunction

Background: There are few reports of cardiac involvement in amyotrophic lateral sclerosis (ALS), even if dysfunction of the sympathetic nervous system influencing the cardiac function has been described. Moreover, there is evidence in the literature that ALS is associated with a favorable cardiovascular risk profile. Here we discuss a possible risk for cardiac involvement in women with bulbar ALS.

Objectives: To report three female patients with bulbar ALS presenting an acute myocardial infarction and one presenting Tako-Tsubo syndrome (TTS), a reversible cardiomyopathy frequently precipitated by a stressful event, with clinical presentation indistinguishable from a myocardial infarction.

Case reports: Patient (Pt) 1(Pt 1), a 52-year old woman, was found to have an asymptomatic subacute apical ischemia and an apical mural thrombus. Considering her compromised nutritional status, 16 days later she underwent a PEG. Shortly after the procedure she presented a serious desaturation and died.

Pt 2, a 77-year old woman was diagnosed as having a silent ischemic heart disease. 12 days later a PEG was performed without complications.

Pt 3, a 62-year old woman, 30 hours after PEG insertion presented a myocardial infarction, treated with Percutaneous Transluminal Coronary Angioplasty (PTCA). She died 14 months later from sudden death.

Pt 4, a 60-year old woman, presented TTS 24 hours after PEG placement with an excellent recovery.

Results: Interestingly, all the four patients were women affected by ALS with bulbar onset. All required PEG as a result of being seriously nutritionally compromised. Pts 1 and 4 had a mild hypertension, well controlled by therapy. Pt 1 was a smoker. Pt 2 had hypercholesterolemia, whilst Pt 3 did not present any major vascular risk factor. None of them had respiratory insufficiency.

Discussion and conclusion: In the general population males are known to have a greater risk for cardiovascular diseases than females, but in a 10-year experience in our ALS Center, we have never observed an acute myocardial ischemia in a male ALS patient, even if we detect subtle ECG alterations in ALS very frequently.

Patients affected by bulbar ALS might have an increased risk of heart ischemia because of a more severe autonomic dysfunction, already reported in ALS with bulbar signs, compared with ALS without bulbar involvement, or through a subclinical intermittent hypoxia. Moreover, the beneficial vascular risk profile associated with ALS and the consequent reduced portion of male patients with vascular risk factors might unmask a gender susceptibility to heart ischemia in this disease.

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P108   MAXIMAL INSPIRATORY PRESSURE (MIP) AND FORCED VITAL CAPACITY (FVC) CORRELATE WITH DISABILITY LEVELS AT TIMES OF ALS DIAGNOSIS

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Keywords: FVC, MIP, disease progression

Objectives: To compare the rate of disease progression and disability in ALS patients who differ in their initial forced vital capacity (FVC) and maximal inspiratory pressure (MIP) values at times of diagnosis.

Background: Respiratory function is a known predictor of individual survival. The AAN practice parameters uses FVC

less than 50% as an indication for NIPPV initiation. MIP is also assessed in current clinical practice. Although, FVC is routinely used as a measure of pulmonary status and selection criteria for clinical trials, others have found MIP to be a more sensitive measurement of pulmonary function.

Methods: A database review of patients diagnosed with definite or probable ALS from 2010–12. Variables studied include age, gender, FVC, MIP, DeltaFS, preslope, ALSFRS and AALS at times of diagnosis and duration of disease. ANOVA analysis was used for comparison of the three groups based upon FVC and MIP at time of diagnosis. Group 1: FVC > 50% and MIP > 60 cm H_2O; Group 2: FVC > 50% and MIP < 60cm H_2O; and Group 3, FVC < 50% and MIP < 60 cmH2O (p < 0.05).

Results: One hundred and five patients were studied (61.4 ± 11.4 years; 48% F; 34% bulbar). FVC and MIP at diagnosis were 78.5 ± 23.7%, −55.7 ± 23.1 cm H_2O, respectively. Group 1 (n = 39) were 33% female and 26% bulbar. Group 2 (n = 54) were 63% female and 35% bulbar. Group 3 (n = 10) were 30% female and 70% bulbar. For Group 4 (FVC < 50%, MIP > 60 cm H_2O, n = 2) the small number limited analysis. There was no difference between groups for rate of progression at time of diagnosis. There was a statistically significant difference between groups for initial AALS score (p = 0.001) and preslope (p = 0.045) with Group 1 having the slowest rate of progression and lower disability score, and Group 3 the fastest progression and higher disability score.

Conclusions: The study confirms FVC and MIP are associated with rate of progression and level of disability at time of diagnosis. The study also shows a significant number of patients with FV > 50% have MIP's < 60 cm H_2O. Additional analysis will be performed looking at MIP, FVC, NIPPV, the actual rate of progression, and survival.

P109   ASYMMETRY OF MOTOR DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS: THE EFFECT OF LIMB DOMINANCE

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Keywords: handedness, limb dominance, clinical phenotype

Background: A key feature of amyotrophic lateral sclerosis (ALS) is asymmetric onset and spread of upper (UMN) and lower motor neuron (LMN) degeneration. The preferential involvement of a particular limb and side of the body may reflect underlying developmental or stress-induced vulnerabilities.

Objective: Our aim was to investigate the relationship between limb dominance and the onset and spread of both UMN and LMN dysfunction in ALS. This expands our prior pilot study (1) by relating onset and spread to the clinical phenotype.

Methods: A structured questionnaire was used to determine the pattern of onset and spread of weakness or disability in 156 consecutive patients with clinically probable or definite ALS, from two tertiary referral centres. The clinical severity of UMN and LMN signs in each limb was also quantified based on clinical examination (2) applied within 12 months of diagnosis or first evidence of limb involvement.

Results: Of the 156 patients, 55% were males and 93% had sporadic disease. Onset site was upper limb in 30%, lower limb in 42% and bulbar in 28%. Upper limb onset of ALS was more likely to occur in the dominant side in right-handed patients. In right-handed patients with initial weakness in a left-sided limb, the direction of disease spread was more likely to remain ipsilateral (p = 0.008). In non-right-handed patients, 67% of upper limb onset was either left-sided or bilateral. The distribution of clinical UMN signs between limbs was also affected by handedness, whereas spread of LMN dysfunction between limbs appeared independent of dominance. There was also a tendency toward early spread of hyperreflexia to limbs unaffected by clinical LMN involvement.

Conclusions: These findings are consistent with an early cortical process underlying spread of disease in patients with ALS who have clinical evidence of UMN involvement. The relationship between handedness and UMN dysfunction may reflect underlying developmental vulnerabilities in the central nervous system, which are exposed in ALS.

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P110   IS THYROID HORMONE ABNORMALITY A PROGNOSTIC FACTOR OF AMYOTROPHIC LATERAL SCLEROSIS?

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Keywords: amyotrophic lateral sclerosis, thyroid hormone, survival

Objectives: To determine the correlation of thyroid hormones and survival in amyotrophic lateral sclerosis (ALS).

Methods: We conducted a prospective cohort study in a group of ALS patients enrolled from May 2006 to January 2013. Level of fasting serum thyroid hormones including free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) were determined at the time of enrolment, and were separated into quintiles based on their distribution of the ALS patients. Survival was calculated as the duration between the onset of first symptom and death

(or tracheotomy, permanent ventilation) or at the time of analysis if alive. Chi-square and Cox regression tests were used to estimate the possible correlation between thyroid hormones and ALS survival.

Results: We enrolled a total of 341 patients with probable or definite ALS, with balanced baseline demographic data among quintiles. The Chi-square test showed that lowered level of serum FT4 was correlated with increased survival in ALS, however, the significance failed to maintain in Cox proportional hazard analysis. There was no statistical difference in survival among patients with different levels of TSH and FT3.

Conclusions: Disturbed level of thyroid hormone does not correlate with longer survival in ALS. There should be no necessity to correct the abnormal levels of thyroid hormone unless it reaches medical needs.

P111   OCULAR MOTOR APRAXIA: AN UNCOMMON EARLY SIGN IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ocular motor apraxia, dementia, ALS-plus

Background: Acquired ocular motor apraxia (AOMA), never described in amyotrophic lateral sclerosis (ALS), is characterized by loss of voluntary control of saccades and smooth pursuit. AOMA is also a characteristic feature of ataxia-oculomotor apraxia (AOA), a neurodegenerative disease caused by mutations in the *APTX* (aprataxin, AOA1) and *SETX* (senataxin, AOA2) genes.

Objectives: To report six patients with ALS also presenting AOMA, two of which at an early disease stage.

Case Reports:

- Case 1: a 62-year-old woman presented an extrapyramidal syndrome at the age of 62 (DaTSCAN was compatible with Parkinson's disease). Three years later she developed ALS and dementia. Progressive fixation of gaze, ocular and eyelid apraxia appeared at the age of 67. Brain MRI showed frontal posterior and parietal areas cortical atrophy; liquoral Aß42 was decreased.
- Case 2: a 77-year-old male was diagnosed as having ALS. Seven momnths after the onset he developed dementia, ocular and eyelid apraxia. Brain MRI showed frontal and parietal lobes cortical atrophy. Liquoral total tau protein was increased. He became comatose in 2 months and died 9 months after onset.

- Case3: a 63-year-old woman, affected by primary lateral sclerosis (PLS), three years after the onset presented ocular apraxia and severe limitation of upper and lateral gaze. Neuropsychological examination revealed an impairing of executive functions and agraphia.
- Case 4: a 71-year-old woman affected by PLS developed ocular apraxia 16 years after the onset of the disease.
- Case 5: a 45-year-old man presented ALS associated with parkinsonism. Ten months later he developed ocular apraxia and a severe slowing of saccades. Brain MRI and neuropsychological assessment were normal. DaTSCAN was compatible with Parkinson's disease. No mutation was found in *MAPT, LRRK2, GRN, ATXN2* (SCA2) and *TBP* (SCA 17) genes. Liquoral total tau protein was normal.
- Case 6: a 57-year-old woman affected by ALS presented ocular apraxia and a severe limitation of upper gaze 6 months after the onset of ALS. A slight impairment of the mnesic function was detected. A brain MRI showed a mild hyperintensity of the corticospinal tracts. CSF analysis and an 18-F-DOPA PET were normal.

Results: ALS with associated atypical parkinsonism was diagnosed in Cases 1 and 5. ALS-FTD and PLS-FTD were diagnosed respectively in Cases 2 and 3. PLS and ALS were diagnosed respectively in Cases 4 and 6. No mutation was found in the analyzed ALS-associated genes (*SOD1, TARDBP, ANG, FUS, C9ORF72,* and *SETX*) and APTX gene. All the patients presented oculomotor apraxia, never reported in ALS.

Discussion and conclusion: Oculomotor function may be impaired in ALS through frontal and parietal lobes involvement, in particular in ALS with cognitive impairment. Early detection of oculomotor apraxia might assume a negative prognostic significance, possibly being associated with an ALS-plus syndrome.

P112   CIRCULATING MIR-1285 AND MIR-29B AS POTENTIAL BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: plasma, microRNA, diagnostic biomarker

Objective: To identify novel biomarkers using microRNA (miRNA) expression profiles in plasma from patients with amyotrophic lateral sclerosis (ALS), and assess their diagnostic values for ALS.

Methods: miRNA expression levels in plasma were determined in five ALS patients and five healthy control subjects using an miRNA microarray from Applied Biosystems Inc. (ABI). Differentially expressed miRNAs in the two groups were screened. The results from the miRNA microarray analysis were validated by real-time quantitative polymerase chain reaction (RTqPCR) in 74 ALS patients and 53 healthy control subjects. The diagnostic performance of miRNAs for ALS was estimated by the receiver operating characteristic (ROC) curve.

Results: Screening using the ABI miRNA microarray revealed that the expression levels of miR-1285, miR-29b, miR-194

and miR-1290 in plasma were different between ALS patients and healthy control subjects. Using larger sample sizes, the results from the validation indicated that the differences in miR-1285 (0.74 ± 0.06 vs. 1.52 ± 0.14, $p = 7.0978 {}^{*}10^{-6}$) and miR-29b (37.63 ± 7.07 vs. 15.25 ± 2.16, $p = 0.0025$) between ALS patients and healthy control subjects were statistically significant. When the relative expression level of miR-1285 was used to diagnose ALS, the area under the ROC (AUC) was 0.734. If the diagnostic threshold was set at 0.9537, both the sensitivity and the specificity were high and the values were 70.3% and 73.6%, respectively. The AUC of miR-29b was 0.669 and the values for sensitivity and specificity were 68.4% and 62.7%, respectively.

Conclusions: The miR-1285 expression levels in ALS patient plasma samples were significantly decreased, while the miR-29b expression levels were significantly increased. These two miRNA species could become diagnostic biomarkers for ALS.

P113   UNTARGETED METABOLOMICS IN CEREBROSPINAL FLUID OF PATIENTS WITH MOTONEURON DISORDERS: DIAGNOSIS PREDICTION TO AN EXTERNAL POPULATION

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Keywords: motor neuron disorders, metabolomics, Nuclear Magnetic Resonance

Background: The diagnosis of Motor Neuron Disorders (MNDs) is often delayed principally due to the absence of reliable diagnostic biomarkers. Metabolomics, a relevant approach to the identification of disease markers, provides metabolic profiles from biological fluids analysis. Nuclear Magnetic Resonance (^{1}H-NMR) is a robust analytical platform to perform such analysis. Although biomarker studies provided relevant findings, robust validation is missing to improve the confidence to diagnosis in clinical practice. The aim of this study was to provide a CSF signature of MND patients by ^{1}H-NMR and to evaluate the performance criteria of such pattern in the prediction of diagnosis from an independent population.

Method: CSF samples, collected at the time of diagnosis suffering from motor neuron conditions and controls were analysed by ^{1}H-NMR. We split the cohort in an 80/20 ratio to provide a training set and a test set. A multivariate analysis (OPLS-DA) was performed on the training set before the prediction diagnosis step on the test set. This experiment was repeated 10 times for assessment of the performance criteria of the diagnosis prediction. Finally, we identified the most discriminating compounds of the metabolome profile.

Results: CSF from 95 MND patients and 85 controls were analysed and discriminating profile between both groups was highlighted. We obtained correct OPLS-DA models characterized by $R^2X > 22\%$, $R^2Y > 93\%$, $Q^2 > 66\%$, and a correct diagnosis prediction with probability mean of 99.31% in the training set. Diagnosis prediction on the test set showed correct performance with 78.9% sensitivity, 76.5% specificity, 78.9% predictive positive and 76.5% predictive negative values. Among the discriminating compounds, we identified threonine, histidine and metabolites of branched chain amino-acids that are linked with pathophysiological ways of MND.

Discussion and conclusion: This analysis confirmed that CSF screening using ^{1}H-NMR is relevant to provide a metabolic signature and to identify promising biomarkers for the diagnosis of ALS. To our knowledge, this is the first metabolomic study in neurological disease based on metabolome profile analysis to predict a diagnosis on an independent validation.

P114   SPORADIC ALS: A SPINAL FLUID PATHWAY DISORDER?

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Keywords: disease progression, hypothesis, cerebrospinal fluid pathway

Objective: To propose an alternative hypothesis for the propagation of ALS.

Background: It is proposed that the onset of ALS is local and that progression is regional (1,2). However, exceptions are notable. For example, recent studies report that 15% of patients with lower limb onset subsequently develop bulbar symptoms 93, 4) and that a large percentage of patients with bulbar onset (38% and 28%, respectively) next develop lower limb symptoms.

Discussion: To account for these exceptions we propose that propagation is mediated by the circulation of spinal fluid. Further, we suggest that ALS represents a CSF pathway disorder preferentially impacting the brainstem and spinal cord which are in close proximity to CSF.

Additionally, the relative sparing of the cerebral hemispheres may represent a dosage effect. Considering the ventricular volume (120 cc), a noxious substance released into the cerebral ventricles would be diluted, relative to the mass of the brain (1400 g), and well tolerated. However, tissue exposure would be enhanced as CSF passed into the fourth ventricle and ultimately into the subarachnoid space surrounding the spinal cord. Accordingly, the brunt of the disease would be borne by neurons and non-neuronal cells in proximity to the CSF pathway. Local variation could result from obstructions to the flow of CSF or other factors. But established locally, contiguous spread would proceed as described (5, 1, 3, 4).

Several disparate clues are compatible with our assumption. Firstly, absorption of CSF from the arachnoid villi decreases with age (6). This offers a possible explanation for the increased incidence of ALS in older persons. Secondly, a number of molecules, including proteins and oligonucleotides, are robustly taken up by motor neurons and non-neuronal

cells from the CSF (7). Finally, CSF from ALS patients is toxic to motor neurons in *in vitro* models (8).

Conclusions: The notion that ALS represents a CSF pathway disorder leads to several lines of research that could provide insight into the cause of ALS. Firstly, it suggests that the study of CSF may lead to the cause(s) of sporadic ALS. Secondly, it suggests that an effort to identify the sources of potentially toxic molecules that find their way into the CSF may shed further light on the origins and spread of the disease.

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P115   PHASE 2 SELECTION TRIAL OF HIGH-DOSAGE CREATINE (CRE) AND TWO DOSAGES OF TAMOXIFEN (TAM) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: clinical trial, creatine, tamoxifen

Background: Preclinical and preliminary clinical data suggest that high dosages of creatine and tamoxifen may be beneficial for people with amyotrophic lateral sclerosis (ALS). Selection design trials can speed the search for effective treatments by screening multiple drugs against each other and choosing the winner for testing against placebo.

Objectives: To select the best of three treatments (creatine (CRE) 30 g/day, tamoxifen (TAM) 40 mg/day, TAM 80 mg/day) based on safety, tolerability, and efficacy profiles.

Methods: This was a double-blind, randomized, three-arm selection trial, following 60 subjects with ALS from nine centres over 9 months. Subjects were randomized 1:1:1 to: CRE 30 g, TAM 40 mg, and TAM 80 mg. Eligibility criteria include ALS duration less than 36 months and vital capacity $\geq$ 50%

of predicted. The primary efficacy endpoint was mean rate of decline in the ALS Functional Rating Scale-Revised (ALS-FRS-R) score using a random slopes model with adjustment for time from first symptom to diagnosis, limb vs. bulbar onset, and baseline vital capacity (VC). Secondary efficacy endpoints included rates of decline in VC and muscle strength measured by hand held dynamometry (HHD). Missing data were treated as missing at random conditional on the observed data and our model assumptions.

Results: Sixty eligible subjects were randomized. Tolerability: the overall rate of drug discontinuation was higher in the CRE 30g group (64%) compared to the TAM 40 mg (43%) and TAM 80 mg (41%) (p = 0.047). Safety: a total of 16 serious adverse events (SAE) occurred during the study (CRE 30 g: 23%, TAM 40 mg: 14%, and TAM 80 mg 29%) and none was considered to be related to study treatments. Nine participants died (CRE 30 g: 14%, TAM 40mg: 10%, and TAM 80 mg: 24%). The CRE group had a higher rate of gastrointestinal adverse events and two subjects had elevated serum creatinine levels. Efficacy: the rate of decline of ALSFRS-R was numerically slower in the tamoxifen 80 mg group (CRE 30g: − 0.9, TAM 40 mg: − 1.0, TAM 80 mg: − 0.7 point/month) and the rate of decline of VC was numerically slower in the Tamoxifen 40 mg group (CRE 30 g: − 3.4, TAM 40 mg: − 2.9, TAM 80 mg: − 3.3 point/month). The differences in ALSFRS-R and VC rates of decline were not statistically significant. Muscle strength in the upper and lower extremities measured by HHD declined at a slower rate in the tamoxifen 80 mg group followed by tamoxifen 40 mg and creatine 30 g groups (p = 0.002).

Conclusions: Tamoxifen 40 and 80 mg dosages are well tolerated in ALS and treatment with tamoxifen showed a slower decline in muscle strength in a dose-dependent fashion. A future trial is needed to test the efficacy of tamoxifen 80 mg against placebo in people with ALS.

P116   SAFETY OF DEXPRAMIPEXOLE FOR THE TREATMENT OF ALS: RESULTS FROM THE RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY, EMPOWER

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Keywords: dexpramipexole, safety, EMPOWER

Background: In a two-part phase 2 study, dexpramipexole (25–150 mg twice daily) was well tolerated for 9 months or less, with dose-dependent trends in slowing the rate of functional decline and reducing mortality in amyotrophic lateral sclerosis (ALS). EMPOWER is an international, prospective, randomized, double-blind, placebo-controlled phase 3 trial of dexpramipexole in familial or sporadic ALS.

Objective: To evaluate the safety of dexpramipexole in a phase III study of patients with amyotrophic lateral sclerosis

(ALS), the EMPOWER trial, and determine if dose-limiting toxicities were present.

Methods: EMPOWER randomized 942 subjects with familial or sporadic ALS to treatment with dexpramipexole or placebo. Patients (18–80 yrs) with symptom onset 24 months or lesser before randomization, slow vital capacity 65% or more of predicted, and no history of or current neutropenia were included. Patients were randomized (1:1) to dexpramipexole 150 mg or placebo twice daily for 12–18 months. Patients on a stable dose of riluzole for 60 days or more before study start and those not taking riluzole were eligible. Safety was evaluated by monitoring adverse events (AE) and laboratory evaluations throughout the study.

Results: The safety of dexpramipexole observed in EMPOWER was similar to previous studies. Most subjects in the dexpramipexole and placebo-treated groups reported an AE (97% dexpramipexole and 96% placebo). Similar incidences of AEs were observed for both treatment groups with respect to severity; AEs considered moderate or severe (81% dexpramipexole and 80% placebo); AEs considered severe (39% dexpramipexole and 40% placebo); serious adverse events (SAEs) (47% dexpramipexole and 50% placebo); subjects who discontinued study treatment due to an AE (11% dexpramipexole and 8% placebo); subjects withdrawing from the study due to an AE (13% dexpramipexole and 13% placebo). More subjects treated with dexpramipexole 150 mg BID experienced AEs related to study treatment (46%) than those who were treated with placebo (32%). Consistent with previous reports, the incidence of neutropenia was higher in participants treated with dexpramipexole (8%) compared with those who received placebo (2%). The most common adverse events (at least 5% incidence and 2% more than placebo) were constipation, nausea, and weight decreased; insomnia; muscular weakness; cough; dizziness; dry mouth; neutropenia; upper respiratory tract infection; and neck pain. The incidence of deaths in the treatment groups was similar, 19% of subjects in the dexpramipexole group and 22% of subjects in the placebo group.

Conclusion: Dexpramipexole was well tolerated in EMPOWER with comparable overall incidences of SAEs, discontinuations due to AEs, and withdrawals due to AEs between the dexpramipexole and placebo groups. No previously unidentified safety issues emerged compared with the earlier phase 2 study of dexpramipexole. No dose-limiting safety signals were observed.

P117   EMPOWER SUBJECTS WITH EL ESCORIAL DEFINITE ALS EXHIBITED SIGNIFICANT BASELINE DIFFERENCES INDEPENDENT OF DISEASE DURATION AND EXPERIENCED SIGNIFICANTLY WORSE OUTCOMES

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Keywords: El Escorial Criteria, dexpramipexole, EMPOWER

Background: Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease characterized by variable clinical presentation and course, increasing the challenge of both clinical management and clinical research. A baseline diagnosis of El Escorial Criteria (EEC)-definite ALS, involving at least three affected regions, is generally associated with worse outcomes. However, it remains controversial whether this solely represents an advanced stage of disease progression or whether EEC-definite ALS may signal a distinct disease subtype associated with diffuse presentation.

Objectives: Subjects (n = 942) in one of the largest controlled studies ever conducted in ALS, the Phase 3 trial of dexpramipexole in ALS (EMPOWER), were retrospectively analyzed according to their baseline EEC classification to assess differences in disease progression.

Methods: Baseline demographics, ALS history, and clinical laboratory values of subjects with definite ALS (EEC-definite, n = 303) were compared to all other subjects (EEC-not definite, n = 639). In the placebo population only (n = 468), on-study changes in efficacy measures and clinical laboratory values were compared between EEC-definite subject n = 156) and EEC non-definite subjects n = 312).

Results: At baseline, EEC-definite subjects were significantly younger (55.789 years vs. 57.659 years, p = 0.0175) and significantly more likely to be female (40.9% vs. 33.3%, p = 0.0243) than EEC-not definite subjects. EEC-definite subjects entered the study with a significantly faster mean pre-study progression rate, as measured by, average monthly decline on the ALS Functional Rating Scale-Revised (−0.80 vs. −0.71, p = 0.0163); a lower mean baseline ALSFRS-R score (36.7 vs. 38.9, p < 0.0001); significantly reduced mean baseline predicted slow vital capacity (85.6% vs. 90.4%, p < 0.0001); significantly lower mean plasma creatinine (68.4 μm/l v. 71.3 μm/l, p = 0.010). Importantly, there was no difference in mean baseline duration of ALS symptoms (15.3 months vs. 15.2 months, p = 0.6252). Over the course of EMPOWER, EEC-definite placebo subjects had significantly worse outcomes on the primary outcome measure, a Combined Assessment of Function and Survival (CAFS, 468.0 vs. 405.9, p = 0.014) and on ALSFRS-R slopes (−1.37 v. −1.06, p < 0.001), while showing a trend toward increased mortality (19.1% v. 16.1%, p = 0.092). EEC-definite subjects also showed a significantly greater reduction from baseline in mean serum creatinine (−16.4 μm/l vs. −12.5 μm/l, p = 0.0056).

Discussion and conclusion: EEC-definite EMPOWER subjects exhibited significant differences from not-definite subjects consistent with more advanced disease at baseline and more rapid on-study progression, confirming that this classification is associated with worse outcomes. However, EEC-definite diagnosis was not an indicator of the duration of disease in EMPOWER, raising doubts that EEC-definite ALS represents solely a stage of progression and suggesting a potential avenue for identifying a specific ALS subtype associated with diffuse presentation. Significance differences in age, gender, and serum creatinine levels exhibited by EEC-definite subjects suggest additional opportunities for investigation.

P118 CREATININE IS A BIOMARKER OF DISEASE SUBTYPE, DISEASE PROGRESSION AND DRUG RESPONSE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN THE PHASE 3 EMPOWER STUDY

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Keywords: creatinine, dexpramipexole, biomarker

Background: ALS is a progressive, heterogeneous neurodegenerative disease with a highly variable clinical course, including survival ranging from a few months to 10 years or more. Validated ALS biomarkers are badly needed to aid diagnosis, measure and predict disease progression and serve as potential surrogate markers of drug response. Without an effective method for measuring disease burden and drug activity, developing effective therapies in treating ALS continues to be elusive. Changes in creatinine levels have been correlated with disease progression and creatinine may be a promising biomarker candidate in ALS.

Objective: To determine if creatinine levels at baseline and changes in creatinine levels over the course of EMPOWER correlate with ALS classification by El Escorial criteria (EEC) and with dexpramipexole-treatment (dex-treated).

Methods: EMPOWER, one of the largest ALS clinical trials ever conducted, enrolled 942 ALS patients. The primary objective of the study was to evaluate the effectiveness of dexpramipexole 300 mg/day. Baseline creatinine levels were compared for participants with definite (n = 303) and not-definite (n = 639) ALS by EEC. Correlations between change from baseline in creatinine levels and the rate of disease progression as measured by the slope of ALSFRS-R at Month 12 were also evaluated. Finally, the effect of dexpramipexole on changes in creatinine levels through Month 12 (time averaged difference) was also evaluated.

Results: Baseline levels of creatinine in subjects with EEC definite ALS were significantly lower than the EEC not-definite group (EEC definite 68.4 μm/l, EEC not-definite 71.3 μm/l, p = 0.0096). During EMPOWER, the slope of creatinine decline (mixed-effects repeated-measure model) was significantly increased in placebo-treated subjects with EEC definite ALS (n = 156) compared with EEC not-definite ALS (n = 312) at Month 12 (EEC definite, −16.4 μm/l, EEC not definite −12.5 μm/l, p = 0.0056). Treatment with dexpramipexole also significantly reduced the decline from baseline (time averaged difference) in plasma creatinine over 12 months (2.71 μm/l, p < 0.001) compared with placebo in patients with EEC definite ALS. The effect of dexpramipexole on creatinine further increased in the EMPOWER subgroup of EEC definite participants receiving concomitant riluzole (3.16 μm/l, p < 0.001), and the EEC definite, concomitant-riluzole and short-symptom duration (< 18 months) subgroup (4.71 μm/l, p < 0.001) where a near-significant improvement with CAFS (416.7/347.7, p = 0.059) was also seen. The significance of this creatinine-sparing effect increased after adjusting for weight change in dex-treated and placebo-treated participants.

Discussion and conclusion: Baseline creatinine levels and changes from baseline in creatinine levels were significantly correlated with ALS classification by EEC and with disease progression by EEC. Treatment with dexpramipexole also significantly reduced the rate of creatinine decline compared with placebo in EMPOWER, including after adjusting for weight change in dex-treated and placebo-treated participants. The creatinine-sparing effects of dexpramipexole increased with increasing disease severity by EEC classification and symptom duration, and correlated with increasing clinical improvement.

P119 EQUIVALENCE OF HISTORICAL CONTROL DATA BETWEEN LARGE ALS TRIALS: SHOULD WE BE PAYING MORE ATTENTION TO THIS DATA?

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Keywords: clinical trial, historical placebo, PROACT database

Background: Over the past few years, the field of ALS has seen several agents prove ineffective in large randomized clinical trials. These studies followed smaller screening trials that showed promise. A more efficient approach is needed to accelerate the identification of useful agents and exclude ones destined to fail. Gaining trust in historical control data would strengthen earlier studies by providing a trove of information for predicting outcomes.

Objectives: To demonstrate that historical placebo outcomes are stable over time, and predictable, as long as data is controlled for differences in inclusion criteria between different trials.

Methods: We compared mean functional declines between placebo patients from the WALS clinical trial of minocycline (MINO, conducted from 2003–5) and from the Talampanel trial (TAL, 2008–10). The TAL data were provided by the PROACT database. These were the two largest trials with data that used the ALSFRS-R. The studies had different baseline inclusion criterion, to ensure we were comparing identical cohorts, we excluded subjects that did not fit the largest overlapping set of criteria that fit both studies. These included: symptom duration less than 3 yrs, at least 4 months follow-up, vital capacity greater than 70% and FRS respiratory subscale 10 or greater. We compared baseline features, and used a linear mixed-effects model over the entire length of the studies to compare differences in mean slopes of the ALSFRS-R.

Results: We included 159 MINO and 191 TAL subjects. The slope declines were nearly identical: −1.02 per month (−1.14, −0.90) for MINO and −1.00 per month (−1.11, −0.89) for TAL (p = 0.83). We also analyzed the subset of patients with symptom duration of less than 2 yrs, and the mean slope declines were MINO −1.13 per month (−0.99, −1.27) and TAL–1.10 per month (−0.97, −1.22) (p = 0.78). There were also no differences when controlling for the effect of symptom duration on outcomes within each study (using a locally weighted least squares regression of slope on symptom duration). Baseline ALSFRS-R were (39.0 ± 4.4 MIN v 38.9 ± 4.7 TAL). TAL patients had slightly longer symptom duration than MIN (18.4 months v 17.0).

Discussion and conclusion: The mean functional declines from these two studies, conducted 5 years apart, appear to be nearly identical and suggest the rate of disease progression is stable. ALS trialists would benefit from considering historical comparisons which improve power, especially when the data can be trusted by showing it yields predictable results over time. Historical control data can augment small concurrent randomized control trials, which run their own risk of outcomes being subject to random statistical variations. These may lead to overly optimistic efficacy predictions. Estimating the expected range of mean functional decline, based on a given set of inclusion criteria, could have raised a caution flag on early trials of dexpramipexole and TAL where the early placebo control cohorts progressed more rapidly than would be predicted.

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P120   AEROBIC CYCLE ERGOMETER ACCORDING TO HEART RATE RESERVE (HRR) IN AMYOTROPHIC LATERAL SCLEROSIS REHABILITATION

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Keywords: exercise, cycle ergometer

Background: Despite the clinical relevance of muscle weakness in individuals with amyotrophic lateral sclerosis (ALS) the best type of exercise and its effect on rehabilitation in this population are not well understood.

Objectives: To determine the safety and exercise treatment-effect size of submaximal aerobic repetitive rhythmic exercise mediated by cycle ergometer for patients with amyotrophic lateral sclerosis (ALS).

Methods: Ten patients were enrolled. Functional status was evaluated by: ALS Functional Rating Scale-Revised (ALS-FRS-R); percentage of predicted vital capacity (VC); oxygen consumption using Heart Rate Reserve (HRR); Medical Research Council scale (MRC) for upper and lower limb strength. Data were collected at baseline; at the end of 4 week; and after washout period of 4 months. The participants underwent submaximal aerobic repetitive rhythmic exercise mediated by cycle ergometer performed twice every day, for 6 days a week, for 4 weeks.

Results: No dropouts or adverse event were observed after cycle ergometer treatment. Safety measures showed stability in ALSFRS-R score during the study interval. Three patients showed the same score in ALSFRS scale from the beginning, three a variance of 1 point, two 3 points, one 4 points and one a variance of 7 points. Also in MRC scale, nine patients showed stability and only one showed a worsening on average of 1 point, in lower limb. Finally VC showed five patients with the same percentage from the beginning, two showed a worsening of 10%, two a worsening of 14% and one 20% less.

Discussion: Cycle ergometer exercise is tolerated, and safe. The treatment supported work capacity and follow up showed that ALS patients should be treated at least three times every year.

Conclusions: Repetitive rhythmic exercise using cycle ergometer should be evaluated further in larger studies to determine the stability of function in relation to the rate of progression of the underlying ALS.

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P121   THE USE OF EYE-TRACKER-BASED NEUROPSYCHOLOGICAL TESTS FOR THE IDENTIFICATION OF C9ORF72 EXPANSIONS CARRIERS AMONG ALS PATIENTS

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Keywords: C9orf72, neuropsychological tests, eye-tracker

Background: Over the past decade growing evidence supports a continuum between Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). The recently discovered hexanucleotide repeat expansions (RE) in the C9ORF72 gene appears to be the most common genetic cause of familial ALS and FTD, and has also been found in a significant number of apparently sporadic cases (1). A bulbar-onset disease and a more severe cognitive-behavioural impairment have been reported in ALS patients with C9ORF72 expansion (2). These findings highlight the

importance of a combined genetic-cognitive screening in ALS, in order to better define genotype-phenotype associations and improve therapy and care. However, neuropsychological (NP) assessment is problematic in moderate-severe stages of ALS, due to motor-verbal impairment. Eye-tracking (ET) has been preliminarily used in ALS to administrate cognitive testing (3,4).

Objectives: To investigate the use of motor-verbal free NP measures, administered by means of the ET technology, for identifying C9ORF72 RE carriers in ALS patients.

Methods: We enrolled 31 patients with probable or definite ALS according to the El Escorial revised criteria. C9ORF72 RE detection was performed using a two-step protocol including a repeat-primed PCR as already described (5). A short battery composed by the adapted versions of cognitive tests focusing on frontal and executive functions, was administered with the ET; patients also underwent a cognitive screening based on traditional paper and pencil tools (Frontal Assessment Battery; Montreal Cognitive Assessment). Patients were divided in two groups according to genetic status; a linear discriminant analysis (LDA) was used to verify if traditional and ET-based measures were useful to discriminate between groups.

Results: Five patients were found to carry RE, while 26 were wild-type. The LDA showed that 97.3% of original grouped cases were correctly classified with traditional tools and 86.3% with ET-based ones. When considering all measures, the classification correctness increased up to 100%, classifying correctly all cases in the two groups.

Discussion and conclusion: The ET technology is suitable to be employed in moderate-severe stage of ALS; we demonstrated that the use of a short ET-based cognitive battery increases the accuracy in the identification of RE carriers up to 100%, with a specific discriminating power similar to that of traditional measures. This supports the introduction of ET-based cognitive tests in the clinical evaluation of ALS, especially in presence of verbal-motor impairment.

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P122   AN ASSESSMENT OF PAIN REPORTS IN ALS USING THREE LARGE DATA SETS

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Keywords: pain, assessment, physical function

Background: Pain has been reported in 50–70% of patients with ALS. This pain can be due to musculoskeletal causes, cramps, or spasticity. The ALS Specific Quality of Life-Revised (ALSSQOLR) instrument is a validated QOL measure. One item within the measure addresses pain by asking "how much of a problem has pain been in the last 7 days" using a scale of 0 (no problem) to 10 (tremendous problem).

Objectives: To examine the frequency and distribution of patient-reported problematic pain scores.

Methods: A retrospective review was carried out of 3 large research data sets used to collect ALSSQOLR, ALS Functional Rating Scale-Revised (ALSFRSR), and demographic data from ALS patients. This included a multicenter dataset collected at 12 US academic ALS clinics in 2006 (DATA1) as part of a validation study of the ALSSQOLR, and two national survey data sets of ALS patients who completed questionnaires in 2007 (DATA2) and 2009 (DATA3) in surveys comparing those who attended multidisciplinary clinics to those who did not. We created pain problem severity categories based on patient problem ratings: 0 = no problem; 1–3 = mild problem; 4–6 = moderate problem; and 7–10 = severe problem. Pearson product-moment correlations between pain and ALSFRSR were calculated.

Results: There were 389 patients in DATA 1, 381 in DATA2, and 385 in DATA 3. All three data sets had similar distributions of gender (approximately 60% male), site of disease onset (25% bulbar, 75% limb), and mean age (approximately 57 years). Mean ALSFRSR in the 3 sets was: 33/48 (DATA 1), 26/48 (DATA 2) and 28/48 (DATA 3). Mean ratings of pain were 2.2 (SD = 2.7) in DATA 1, 2.7 (SD = 2.9) in DATA 2, and 2.6 (SD = 2.8) in DATA 3. The three data sets show similar distributions of pain report scores: DATA 1 (no problem = 40%, mild = 34%, moderate = 16%, severe = 10%); DATA 2 (no problem = 33%, mild = 36%, moderate = 15%, severe = 16%); and DATA 3 (no problem = 34%, mild = 36%, moderate = 16%, severe = 14%). A negative correlation (p < 0.05) of ALSFRSR and pain was present in all samples r = − 0.16 (DATA 1), r = − 0.15 (DATA 2), and r = − 0.14 (DATA 3).

Discussion and conclusion: Three distinct data sets collected over 3 years report similar patterns of pain assessment by ALS patients. Poorer physical function was associated with more problematic pain, consistent with previous studies. While on average, ALS patients report pain to be a mild problem, 30% report that pain is a moderate to severe problem. Additional large, prospective studies to characterize the nature, distribution, and severity of pain in ALS would aid in the development of systematic approaches for pain management.

P123 USE OF VETERANS SPECIFIC ACTIVITY QUESTIONNAIRE [VSAQ] TO ESTIMATE EXERCISE TOLERANCE IN AMBULATORY AMYOTROPHIC LATERAL SCLEROSIS (AMBALS) PATIENTS

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Keywords: exercise tolerance, physical actvities, metabolic equivalent

Background: Many ALS patients want to know whether they can exercise and how much exercise they should do. Exercise prescription should be tailored to each patient's tolerance and functional capacity. The cardiopulmonary exercise test (CPET) is an established procedure used to assess an individual's tolerance to exercise by directly measuring peak oxygen uptake. The application of CPET in an ALS multidisciplinary clinic would be time consuming, requires elaborate equipment, and technical training. Physical activity symptom questionnaires have been used to estimate patient's tolerance and functional capacity for the development of an exercise prescription. The VSAQ (1) is validated instrument used to estimate exercise capacity that consists of physical activities (PA) listed in progressive order according to their energy demand estimated by metabolic equivalents (METs). The MET values associated with each activity are in general agreement with the American College of Sport Medicine Compendium of Physical Activities (2). One MET is defined as the amount of oxygen consumed at rest, generally equal to 3.5 ml/kg/min. Therefore, the numbers of METs express the energy cost of PA as a multiple of the resting metabolic rate.

Objective: To determine whether a questionnaire-based method using the VSAQ is a practical tool to estimate exercise capacity in ambALS.

Methods: Thirty ambALS patients completed the VSAQ. Patients were instructed to identify which PA would be associated with cardiovascular symptoms (fatigue, chest pain, or shortness of breath). The ALS Functional Rating Scale-Revised (ALSFRS-R), forced vital capacity (FVC), 25-foot walk test (25FWT), timed-up-and-go (TUG) were performed; 19 patients completed a 6 minutes walk test (6MW). Pearson's correlations were used to test for linear relationships between the VSAQ (predicted MET) with the other outcome measures.

Result: Significant correlation was observed between METs and the ALSFRS-R ($r = 0.578$, $p \leq 0.001$), mainly due to its correlation with the gross motor subscore ($r = 0.65$, $p \leq 0.001$), but not with bulbar, fine motor, or respiratory subscores. AmbALS patients with higher MET were faster in TUG and 25FWT ($r = -0.625$, $p \leq .0.001$ and $r = -0 - .484$, $p \leq .0.001$ respectively), and walked further in 6MW ($r = 0.56$, $p < 0.001$). No correlation was found between METs and FVC.

Discussion and conclusion: VSAQ is a practical tool to estimate exercise capacity of ambALS patients and may be used for

safe exercise prescription to define PA that might be tolerated by the ambALS patients. Estimated exercise capacity of ambALS patients predicted by VSAQ correlated with timed functional measures of motor function and ALSFRS-R, but not with FVC or the respiratory and bulbar subscores of the ALSFRS-R.

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P124 VALUES: A NATIONAL MULTICENTER STUDY EVIDENCING GENDER DIFFERENCES IN THE BEHAVIORAL VARIANT OF FRONTOTEMPORAL LOBAR DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: bvFTLD, gender, HRT

Background: Behavioral variant Frontotemporal Lobar Degeneration (bvFTLD) has three recognized subtypes that reflect different patterns of deterioration: Disinhibited (DIS), Apathetic (APA), and Stereotypic (STE) subtypes (1). We hypothesized that with emergence of bvFTLD, female gender would be associated with a more anterior and bilateral pattern of neurodegeneration, characterized by a less florid presentation.

Objectives: To investigate gender differences in incidence rates and pattern of symptomology of the three bvFTLD subtypes in the ALS population as they emerge.

Methods and materials: One hundred and six subjects (53 male) from 14 ALS clinics were evaluated cross-sectionally with the caregiver rating Frontal Behavioral Inventory. Gender groups were equivalent for IQ, while females were older (male, mean = 57.1, female, mean = 62.5; p = 0.013) and males more educated (male mean = 15.5, female mean = 14.3; p = 0.018). We evaluated subtype gender differences by independent t tests. We evaluated gender associated traits by Spearman rho correlational analysis.

Results: A significantly greater proportion of males evidenced the DIS subtype (p < 0.001), with three of the five inclusive traits evidenced in males to a statistically significantly greater degree: loss of insight (p < 0.001); Jocularity (p = 0.009), and Impulsivity (p = 0.005). For the female subgroup, incidence rates for moderate-severe levels of concreteness (98.7%) and perseveration (79.8%) were markedly lower, while personal neglect (120.2%) was markedly higher. For concreteness, a significant correlation was found for the male subgroup with impulsivity (p < 0.001), while a

significant relationship was found for the female group with indifference (p < 0.001). For the female subgroup, significant relationships were also detected between personal neglect and all other traits evaluated: Indifference (p < 0.001); impulsivity (p = 0.013); jocularity (p = 0.013); concreteness (p = 0.012).

Discussion and conclusion: From a review of the human neuroimaging literature across a broad spectrum of related disciplines, and based upon our pattern of findings, we have generated a model of FTLD onset consequent to disruption to the ventral tegmentum – ventral striatum – forebrain dopaminergic neural system (2). We propose a novel therapeutic approach applying the gonadal steroid hormones to attenuate the FTLD prodrome in relatively young ALS individuals in or just post-menopause or andropause (2).

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P125   CLINICALLY MEANINGFUL CHANGE ON THE ALSFRS-R

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Keywords: ALSFRS-R, outcome measure, clinical research

Background: ALS is heterogeneous in location of onset and rate and pattern of spread. The ALS Functional Rating Scale-Revised (ALSFRS-R) is an outcome measure with four functional domains focused on bulbar, respiratory and gross and fine motor function. A clinically meaningful change on the scale is not uniformly defined and each functional domain impacts patients' independence and function differently. Only one prior study (1) assessed the clinical relevance of percentage changes in decline of ALSFRS-R slope. There are no evaluations of clinical meaningfulness of change in each functional domain.

Objectives: To assess the clinically meaningful threshold of change on the ALSFRS-R functional domains in the opinion of ALS expert providers.

Methods: Thirty-eight ALS experts, all members of the Northeast ALS Consortium (NEALS) were gathered in person to evaluate the clinical meaningfulness of changes in functional domains on the ALSFRS-R. Participants evaluated 48 questions/scenarios and rated changes as not-, minimally-, moderately-, or very clinically meaningful. Questions were presented as either ALSFRS-R score changes in each functional domain or changes presented as clinical passages corresponding to changes in the ALSFRS-R. Changes were in either individual functional domains or across multiple domains.

Results: When changes were presented as ALSFRS-R scores, a slight majority of providers rated a 2-point change moderately or very clinically meaningful (MVCM) in the gross-motor (58% of respondents) and bulbar (53%) domains; in the fine-motor domain, a minority of respondents rated 2 points as clinically meaningful (42%). In the respiratory domain, a clear majority rated a 2-point change as MVCM (70%); Three-point changes (but not two point changes) were rated as MVCM by the majority of respondents when the changes involved multiple functional domains. When offered clinical passages, the majority of experts rated the following changes as MVCM: ≥ 2 points (bulbar and respiratory domains) and ≥ 4 (fine motor domain). The majority of respondents rated a change of 3 points, but not 4 points, as MVCM in the gross motor domain.

Discussion and conclusion: ALS experts rated changes similarly whether presented as cases or numerical changes in the ALSFRS-R. Interestingly, smaller changes in bulbar and respiratory functional domains were considered more clinically relevant than in other domains. This suggests that perhaps changes in the total ALSFRS-R score should be adjusted for the domains affected prior to statistical analysis in trials. This could impact power calculations and trial results, providing trial analyses that are more clinically meaningful and better account for disease heterogeneity.

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P126   STATISTICAL CONSIDERATONS IN THE PRIZE4LIFE DATA MINING CONTEST INVOLVING PREDICTING ALSFRS

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Keywords: data mining, clinical prediction, crowd-sourcing

Background: Two non-profits, PRIZE4LIFE and DREAM, organized a contest for the development of a predictor of a patient's outcome to guide clinical management and reduce the size of clinical trials.

Objectives: We describe the statistical issues in the design and evaluation of this contest and report some of the results.

Methods: Contestants were given a training data set to develop predictors which were tested on a test data set. There was an intermediate round where contestants could repeatedly test their solution on a validation data set. These data sets were drawn from the large PROACT database of data from ALS clinical trials. The contestants used data from a patient's first three months to predict the change in ALSFRS in the next nine months. The root-mean-squared-error was used to compare the solutions.

In this study, we required that predictions be computable from an individual patient's data, rather than the entire data

set. The mean square error from the test data set for each contestant is unbiased. But the best of these mean squared errors of would be biased if the difference between leading entries were due to chance. This bias was estimated by taking bootstrap samples from the test data set, using each bootstrap sample to determine the winner and testing the winner's solution against the observations that were not in the sample. To assess the usefulness of the prediction we simulated clinical trials by adding a treatment effect to the elements of the test data set.

Results: A total of 1073 participants entered the challenge, 37 submitted algorithms, and 12 entered the final round. Two finalists were disqualified because their predictions depended on the entire data set. The six best contestants were not significantly different. Two contestants were selected as winners. The winning entries reduced the variance of ALSFRS changes by 17%. The extent of over-fitting was minor.

The use of the top predictor could reduce trial sample size by 22%. Although various laboratory tests were available for prediction, the past disease progression was identified by all predictors as the best predictor for the future progression

Discussion: The number of contestants participating in the final round may have been reduced by the difficulty of manipulating the data set and by the leader board. Many of the statistical problems did not materialize. It will be necessary to reduce the length of the lead in period if we intent to use these predictions to reduce the sample size of clinical trials. Since the potential reduction in sample size is only 20% large clinical trials are necessary in ALS.

P127   EVALUATING THE PENN STATE HERSHEY COMMUNICATION AND TREATMENT PREFERENCE TOOL

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Keywords: treatment goals, communication, evaluation

Background: As ALS progresses, patient's goals of treatment often change. An understanding of these goals is essential for framing treatment decisions. Patients often believe that completion of an advance directive is by itself sufficient and may fail to realize that conditions anticipated by advance directives rarely develop in ALS, and that advance directives are only one piece of the planning process. We developed the *Penn State Hershey ALS Communication and Treatment Preference Assessment* (PSHCTPA) to document patient's completion of legal documents related to end of life, preferences for information, services, and treatments for ALS, and thoughts about living well with the disease.

Objectives: To conduct a pilot trial of the PSHCTPA in order to permit evaluation of this instrument by patients with ALS and by ALS clinic staff.

Methods: During a 3-month period, the PSHCTPA and a research summary sheet were mailed to patients one month before their ALS Clinic appointments. The clinic physician and nurse reviewed the form with the patient in clinic. Both patients and ALS clinic staff completed evaluations of the instrument.

Results: The PSHCTPA was sent to 95 ALS clinic patients, 27 of whom completed it. Mean age was 65 years (SD 11.1), mean ALSFRSR 26.9 (SD = 11.1), and average disease duration 73 months (range = 13–265). Fourteen patients completed anonymous evaluations of the form. 85% reported no difficulty completing the form. 86% indicated that completing the form helped them communicate their wishes to the ALS clinic team and all thought that use of the form would improve the care received from the ALS team. 79% felt that communication and treatment preference assessment was an important topic to discuss. When asked how often the form should be reviewed in the ALS clinic: 36% reported "at every ALS clinic"; 36% reported "every other ALS clinic"; 7% reported "yearly"; 21% indicated "at the discretion of the ALS team". Clinic staff reported that the form was useful in clarifying treatment goals, providing an opportunity to give resources on advance directives and other legal documents, and discuss mechanical ventilation and other end-of-life goals and decisions.

Discussion and conclusion: Patients found the form to be useful as part of their ALS care. Staff found the form to be helpful, particularly in clarifying treatment goals. Engaging with patients consistently and in a manner sensitive to their personal style of communicating and receiving end-of-life care is difficult in a busy ALS clinic. This form encourages patients to discuss end-of-life care as a routine part of their ALS clinic visit, enhances understanding of treatment goals by patients and the health care team, and may improve care by facilitating discussion of these goals.

P128   FIBEROPTIC ENDOSCOPY EVALUATION OF SWALLOWING IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: deglutition, deglutition disorders, endoscopy

Background: An early diagnosis of swallowing disorders in patients with Amyotrophic Lateral Sclerosis (ALS) is important to allow patients to have a proper therapeutic approach according to clinical symptoms. The endoscopic evaluation of swallowing is an image examination that provides indirect informations about oral phase and pharyngeal phase of swallowing, and also laryngeal and pharyngeal structures in relation to motility and sensitivity.

Objective: To evaluate the swallowing of patients with ALS using fiberoptic endoscopic evaluation of swallowing (FEES).

Methods: A transversal historical cohort study in 11 patients with ALS; Six male (54.5%) and Five female (55.5%); median age 61.7 years. The period of disease reported was

26.0 ± 14.6 months and the period of diagnose of the neurological disease was 13.9 ± 12 months. These patients underwent anamnesis and FEES.

Results: All patients presented bulbar symptoms, 81.8% (9) were feeding orally and 18.2% (2) used alternative methods via nonexclusive feeding (nasogastric tube). All patients had, at least, one phase of swallowing altered, but only 72.7% had swallowing complaints. The preparatory phase was altered in 63.6% and the oral and pharyngeal phases in 100% of individuals, despite of the consistence of the food. The laryngeal penetration or tracheal aspiration was observed in 90.9% of the patients during the pharyngeal phase of swallowing of liquid.

Discussion: The endoscopic evaluation of swallowing can assess every food consistency and can still be carried out in bedridden patients or even those using alternative feeding devices and respiratory devices, increasing its options of diagnosis and continuance. The premature spillage is more often with thin liquid texture, it is the major cause of tracheal aspiration even in the early stages of the disease with light changes in the oral muscles. The laryngeal penetration or tracheal aspiration of food can indicate inability to control food bolus, a reduction in the elevation of hyoid–larynx complex, a decrease in the contraction of the pharyngeal constrictor muscle and or presence of residues in valecule and piriform recesses, exposing the patient to the risk of bronchoaspiration.

Conclusion: The oral and pharyngeal phases are the most frequently altered in patients with ALS. The laryngeal penetration or tracheal aspiration occurred more frequently during pharyngeal phase of swallowing of liquid. Despite the absence of any swallowing complaint, the deglutition disorder is frequent in ALS, so that it is mandatory to investigate the dysphagia in these patients.

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P129   PRIMARY LATERAL SCLEROSIS: ARE THE PRINGLE CRITERIA STILL VALID?

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Objective: Since primary lateral sclerosis (PLS) is mainly a clinical diagnosis validity of its diagnostic criteria is crucial. For 20 years the Pringle criteria has been commonly used for this purpose; however, the validity of the Pringle criteria has never been investigated.

Methods: In this study, we reviewed the Pringle criteria in the context of our database of 76 patients who were diagnosed in specialised motoneuron outpatient clinics.

Results: We found that the Pringle criteria cannot be applied to most of the patients. For example, the beginning of the disease, PLS patients frequently showed an asymmetrical clinic presentation. Further observations have been made, some of them, based on the new scientific insights which have been discovered in the last years, contradict the Pringle criteria and will be discussed.

Conclusion: Based on diagnostic assessment of our large cohort of PLS patient, we suggest a new diagnostic pattern based on our results and considerations.

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P130   AN EVALUATION OF NEWLY DIAGNOSED PATIENT NEEDS: LESSONS LEARNED FROM PATIENTS AND FAMILIES

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Background: The ALS Association Greater Philadelphia Chapter patient service staff sought to understand the needs of newly diagnosed patients and their families during the period shortly after diagnosis. Many services and programs are available, but there is no information on the usefulness of these services to patients and families.

Objectives: To seek information about coping behaviours and services utilized by patients and family members in the first 6 months following diagnosis of ALS.

Methods: An evidence based practice model was utilized. A review of the literature did not identify research addressing these specific needs during the 6-month period following diagnosis. A focus group of expert clinicians in ALS care (social workers, a nurse, and a counsellor) developed a survey. Persons with ALS (PALS) diagnosed in the previous 2 years and their family members were contacted by email or via the postal service and asked to complete the survey.

Results: Four hundred and seventeen people were contacted by conventional mail with a response rate of 22.2%, whereas 278 were contacted by email with a response rate was 23.7%, giving a total response rate of 22.8%. 50.3% of respondents were PALS and 49.7% were family members. There were no significant differences between the PALS or family groups on any items. Responses were therefore combined. The most helpful services were the first clinic visit (79%) information (59%) and support groups (22%). 80% sought information outside of the physician appointment, with the internet as most common source. Only 31% wished to meet others during this period. Responses to what helped coping during this period were, from most to least frequent responses: (1) Friends and family; (2) ALS Clinic visit; (3) Gathering information; (4) Spiritual life; (5) Own perspective; and (6) Avoidance. When asked what else would be helpful, only 50% responded and those responses were split between an in-person, or web-based seminar, 'webinar'. Only 2.2% reported attending a newly diagnosed seminar, responding that they did not do so because it was inconvenient (43%); they were not aware (38%); or they were not ready (33%).

Discussion and conclusion: PALS and their families have similar coping experiences and behaviours in the first 6 months following diagnosis. The primary coping strategies focus on information seeking, expert medical care at a multidisciplinary clinic and connecting with others. The results of this survey are being used to evaluate and guide services for the ALS chapter.

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P131   FALSE POSITIVE DIAGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS: A TWO-YEAR RETROSPECTIVE COHORT STUDY IN TURIN

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Keywords: ALS, misdiagnosis, atypical features

Aims: To verify how many cases of alleged ALS referred to the Turin ALS Center for diagnosis confirmation received a different diagnosis, and to assess their characteristics.

Methods: The Turin ALS center is the reference center for ALS in Piedmont region. All subjects referred to the center from 1 January 2011 to 31 December 2012 with an alleged diagnosis of ALS have been included in the study. All subjects underwent a complete diagnostic work-up, including brain and cervical MRI scan, neurophysiological examinations and other exams when deemed necessary, according to EFNS guidelines for ALS.

Results: Out of a total of 390 patients referred to our ALS Center, 296 received a final diagnosis of ALS and 27 a diagnosis of non-ALS MNDs (ie progressive muscular atrophy, primary lateral sclerosis, monomelic MND, and post-polio syndrome). Sixty-seven patients (17.2%) were reclassified as having other disorders, the most common being cervical myelopathy and myopathies. The 296 patients meeting the eligibility criteria included 124 women and 172 men and had a mean age at onset of 63.1 years (men: 62.4; women: 64.2). The presentation was spinal in 194 cases (65.5%) and bulbar in 99 cases (33.5%). The 27 patients meeting a diagnosis of non-ALS MND included 13 women and 14 men; the mean age at onset was 54.7 years (men: 58.0; women: 53.3). The 67 patients affected by other diseases included 42 men and 25 women; their mean age at onset was 53.7 years (men: 51.8; women: 56.9). The most frequent diagnoses were cervical myelopathies and extrapyramidal disorders (men) and myopathies (women). The male/female distribution showed no significant difference among the three groups (p = 0.61). Patients not confirmed as affected by ALS were significantly younger (p < 0.05) and showed generalized asthenia (p < 0.05), atypical symptoms (p < 0.05), and symmetrical disturbances (p < 0.05) as presentation symptoms.

Conclusions: In our series, 17.8% of patients referred as ALS were reclassified to be affected by other disorders, higher rate than that previously reported in other population-based studies. In our study, misdiagnosis was more frequent in young men and the most common confounding symptom was symmetrical weakness at the lower limb muscles. In the absence of a definitive diagnostic test, the diagnosis of ALS is clinically based. All suspected ALS patients should undergo electrophysiological testing by an experienced neurophysiologist while imaging is required in some presentations, such as limb-onset without bulbar involvement, or symptoms and signs isolated to the bulbar region. Failure to progress as expected or the development of atypical features should prompt reconsideration of the diagnosis in order to exclude treatable ALS mimics.

P132   PHENOTYPE AND GENOTYPE STUDIES OF ALS CASES IN ALS-ASI FAMILIES

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Keywords: phenotype, co-occurrence, SMA

Background: Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are the most prevalent motor neuron affections in adulthood and childhood, respectively. Since both conditions are characterized by lower motor neuron degeneration and several reports mentioned occurrence of both disorders within the same family, a common genetic factor to both conditions was suspected. Currently, there is no evidence for a main role of SMN genes in such co-occurrence and the phenotype of these cases has never been well described.

Objectives: To determine the phenotype and genotype of these ALS cases.

Methods: We performed a retrospective study on 11 ALS cases from nine families in which cases of ALS and SMA co-exist. For all ALS cases, gender, age of onset, site of onset and duration of the disease were collected. After written informed consent, SOD1, TARDBP, FUS, C9ORF72 and VAPB genes were screened in the samples. A quantitative SMN gene copy number was also performed in all cases.

Results: In our cohort, there were 8 familial ALS (FALS) comprising 6 men and 5 women, with a mean age of onset of 58.5 years. Site of onset ranged from bulbar (1 case), upper limb (1 case) and lower leg (9 cases). Seven cases died after a mean duration of disease was 18.3 months, the remaining four were still alive after a median duration of 72 months. Three mutations were found in three different pedigrees: two in the SOD1 gene (G147N, E121G) and one in the c9ORF72 gene (number of GGGCGG repeat > 30). 3 out of the 11 had abnormal SMN1 copy numbers.

Discussion and conclusion: Since the phenotype of these cases matched for that of classical FALS, theses co-occurrences, more frequent than expected, might support searching for other genetic factors and strengthens the oligogenic hypothesis of MND.

P133 CLINICAL AND MOLECULAR CHARACTERIZATION OF A COHORT OF PATIENTS WITH DISTAL MOTOR NEURONOPATHY

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Keywords: distal SMA, lower motor neuron disease, diagnosis

Background: Within the vast spectrum of Lower Motor Neuron Syndromes, the distal hereditary motor neuropathies/neuronopathies (dHMN) represent a genetically heterogeneous group of diseases characterized by distal muscle weakness and atrophy, and no or minor sensory abnormalities. However, 80-90% of patients with dHMN carry a mutation in an as-yet undiscovered gene.

Objectives: Here we present the clinical, pathological and molecular findings of patients presenting with motor neuropathy evaluated for the four most frequent genes responsible for dHMN: small heat shock protein 1 and 8 (*HSPB1* and 8), glycyl-tRNA synthetase (*GARS*), Berardinelli-Seip Congenital Lipodystrophy type 2 gene (*BSCL2*), and senataxin (*SETX*).

Methods: For all patients, demographic characteristics, family history, disease duration, and site of onset were recorded. The contribution of additional diagnostic tests, including sensory or motor nerve biopsy and brachial plexus magnetic resonance imaging (MRI), has also been considered.

Results: A total number of 37 unrelated patients were evaluated (Males: 13 and Females: 24). Family history was positive for five patients, while consanguinity was observed in three patients. Mean age at onset was 33.9 years ± 20.7; mean disease duration was 12.9 years ± SD: 11.3. The site of onset was at the upper limbs for 9 patients (24.3%) and lower limbs for 28 patients (75.7%).

The molecular analysis identified mutations in four patients in three different genes. Of note, two mutations in *SETX* were identified in two young males previously diagnosed as multifocal motor neuropathy.

Moreover, we found a previously reported mutation in the *BSCL2* gene in a 13-year-old boy presenting with progressive upper limb weakness. We also identified a previously reported mutation in the *HSPB1* gene segregating in a large Sardinian pedigree with a variable clinical phenotype ranging from CMT2 to dHMN and spastic paraplegia. Interestingly, the following alternative diagnosis was formulated at subsequent follow-up for five patients: inclusion body-myositis (supported by motor nerve and muscle biopsy), diffuse sporadic spinal muscle atrophy (supported by motor nerve biopsy), flair-arm syndrome (two patients), and Hyrayama disease (supported by brachial plexus MRI).

Discussion and conclusion: Our case record confirms a similar percentage, of detection-rate for mutations in dHMN genes, when compared to the literature. Moreover, we suggest that genetic screening should be considered in the context of a complex diagnostic approach, in order not to overlook other possible diagnoses.

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P134 THE CLINICAL CHARACTERISTICS OF ALS PATIENTS IN DIFFERENT ETHNIC GROUPS

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Keywords: ethnic groups, disease progression, prognosis

Background: The incidence and prevalence of ALS has been reported to be lower in non-White ethnic groups compared to Caucasians in the United States (1,2). Other studies have suggested that there is a difference in the mortality in non-White ALS patients compared to that of Caucasian ALS patients (3). The cause for these differences between ethnic groups is unknown and may be related to variations in the genetic background. The clinical characteristics that may reflect the genetic variability in non-White ALS patients have not been well described.

Objective: The aim of this study is to compare the clinical characteristics of ALS patients in different ethnic groups.

Method: A retrospective chart review was conducted for all patients diagnosed with ALS at University of Maryland ALS Clinic between 2007 and 2013. Information extracted include: ethnicity, gender, revised El Escorial diagnostic criteria classification at the time of diagnosis, date of symptom onset, date of diagnosis, site of symptom onset, clinical measures of motor function, ALSFRS-R, forced vital capacity, and riluzole use. The rate of disease progression was calculated as follows: (ALSFRS-R score at first time point evaluation – ALSFRS-R score at last time point)/follow-up interval in months.

Results: The medical records for 86 ALS patients were reviewed. There were 72 (83.7%) non-Hispanic Caucasian patients and 14 (16.3%) non-White patients. There were 12 African American patients, one Asian patient, and one Hispanic patient. There were 39 (45%) women and 47 (55%) men. Fifty-five (64%) patients had limb onset ALS and 29 (33.7%) patients had bulbar onset ALS. The frequency of bulbar onset ALS was greater in Caucasian patients compared to that of non-White patients ($p = 0.048$). The rate of disease progression was similar in

non-White ALS patients compared to Caucasian ALS patients. There were no other significant demographic or clinical differences between non-White and Caucasian ALS patients.

Conclusion: Non-White ALS patients may be underrepresented in a typical academic medical center ALS clinic. Most non-White ALS patients have limb onset ALS which may confer a more favourable prognosis and partly account for the lower mortality in this group of patients.

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P135 URIC ACID IN AMYOTROPHIC LATERAL SCLEROSIS: NO EFFECT ON OUTCOME IN A POPULATION-BASED SERIES

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Keywords: ALS, uric acid, prognosis

Background: Elevated uric acid levels have recently been found to be associated with slower disease progression in several neurodegenerative disorders (Parkinson's disease, Huntington's disease, and multiple system atrophy). There are limited and uneven results in ALS.

Methods: ALS patients resident in Piemonte and seen between 2007 and 2011 have been considered for the study. Blood biochemical factors studied included glucose, creatinine, creatine kinase, uric acid, cholesterol, triglycerides, HDL, LDL, albumin, bilirubin, TSH, fT3, fT4, and electrolytes (Na, K, Cl, Mg, and Fe). Serum samples were obtained at the time of diagnosis and immediately processed. The median time from onset to diagnosis was 8 months. All biochemical analyses were performed in the same laboratory. The mean outcome measure was survival time, calculated as tracheostomy-free survival. Levels of biochemical factors were stratified into quartiles, based on the distribution of the results. Since some biochemical values significantly differed between genders, stratification was performed separately for men and women. In Cox multivariable analysis (stepwise backward), clinical factors known to be related to survival were considered as covariates (ie age at onset, site of onset, FVC, ALSFRS-R score, BMI, diagnostic delay, and riluzole use).

Results: A total of 312 patients were included (169 men and 143 women; mean age at onset 65.5 years $\pm$ 10.3. Uric acid levels were higher in men than in women (5.3 $\pm$ 1.3 vs. 4.4 $\pm$ 1.2, p $=$ 0.0001). In univariate analysis, uric acid levels were not correlated with survival both in men and in women, although men with the highest quartile of acid uric levels showed a 2-year longer median survival than all other quartiles. In Cox multivariable analysis, uric acid level did not prove to be an independent prognostic factor both in men and in women.

Discussion: We have found no evidence of a specific effect of uric acid levels on ALS outcome in a population-based series of ALS patients.

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P136 AMYOTROPHIC LATERAL SCLEROSIS (ALS) ESTIMATES FROM NATIONAL DATABASES IN THE UNITED STATES FROM 2001 TO 2010

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Keywords: ALS registry, national registry, incidence, prevalence

Background: The uncertainty about the incidence and prevalence of amyotrophic lateral sclerosis (ALS) in the United States, as well as the lack of knowledge about the role of environmental exposures in the etiology of ALS, has created a need for structured data collection through a national ALS registry. In 2008, a law providing for the creation of a national ALS registry was signed. From 2001 to 2005, the Agency for Toxic Substances and Disease Registry (ATSDR) conducted four pilot projects to determine the feasibility of creating a national ALS registry, which showed that approximately 80% of ALS patients can be found through national databases. Nevertheless, several different methodologies would be needed for identifying a large portion of individuals with ALS in the U.S. Therefore, in 2009, ATSDR began implementation of the National ALS Registry using a two-pronged approach to help identify all U.S. cases of ALS. The first approach utilizes existing national administrative databases to identify prevalent cases, based on an algorithm developed through the pilot projects. The second approach, implemented in the fall of 2010, uses a secure web portal to identify cases not included in the national administrative databases. This approach allows patients to self-identify and enrol in the ALS registry and take risk factor surveys.

Objectives: To present preliminary ALS estimates from national databases in the United States.

Methods: We sought to identify U.S. residents with ALS in Medicare, Medicaid, Veterans Health Administration (VHA), and Veterans Benefits Administration (VBA) databases, for the years 2001–2010. Records were searched and identified in Medicare, Medicaid, and VHA for ICD-9 codes of 335.2–335.29. VBA records were searched and identified for codes of 8005 (Progressive Bulbar Palsy), 8017 (ALS), and 8023 (Progressive Muscular Atrophy).

Results: A total of 147,889 individuals were identified across the four national databases. Medicare contributed the largest number of individuals in all three categories, and contributed the most to identified ALS individuals (95%). The total number of individuals identified as ALS was 36,547, which is 24.7% of the total number of individuals identified with any MND.

Discussion and conclusion: This is the first effort to identify ALS cases for a national registry in the United States. Although the number of individuals identified via the national administrative databases is not a prevalence estimate, it does indicate that a large portion of ALS individuals can be identified for the National ALS Registry by using the national administrative databases selected.

P137 CLINICAL CHARACTERISTICS OF AFRICAN AMERICAN PATIENTS WITH ALS, THE NORTHWESTERN ALS/MDA CLINIC EXPERIENCE

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Keywords: African American, ethnicity, slow progression

Background: Prior whole genome association studies in ALS suggested that the ethnic background may modulate the genetic risk of ALS. There are reports of lower incidence of ALS among African American, Asians or Hispanic compared to those of Caucasian. However, studies that describe the phenotypic characteristics of ALS in these populations are lacking.

Objective: To describe the clinical characteristics of African American patients with ALS/MND.

Methods: Retrospective chart review of ALS/MND patients of African American descent who attended either the ALS or MDA clinic at Northwestern University in Chicago between 2004 and 2013.

Results: We identified 32 African American new patients with ALS/MND among the 900 new patients with ALS/MND seen during that time period. The male to female ratio was 1.90–1.00, the average age of onset was 60.2 years and the average disease duration was 5.25 years. 62.5% of the patients had limb onset disease whereas 28% had bulbar onset disease and about 1% had diaphragmatic onset weakness. Only 34% of the patients had classic ALS. When we looked at the proportion of patients with slow disease progression, we found out that about 31.2% of the patients survived beyond 5 years. 55% of the patients had a lower motor neuron predominant phenotype and 60% of the patients had a spinal ALS phenotype. 15% of patients were lost of follow up and 9% of the patients chose mechanical ventilation.

Discussion: The clinical characteristics of African American patients with ALS seem to be different than Caucasians. Classical ALS was rare. Spinal form of ALS, lower motor neuron predominant disease and slow progression seemed to be the predominant features of this subgroup of patients. The attitude toward care and end-of-life decision with a higher rate of mechanical ventilation and loss of follow-up appear to distinguish this group as well.

Conclusion: African American patients with ALS may constitute a unique subgroup of patients that need to be studied in more detail. Ethnic background ought to be looked at more carefully during the design of clinical trials.

P138   THE EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS IN NEW HAMPSHIRE, USA, 2004–2007

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition with both genetic and environmental risk factors. Epidemiology can provide clues to disease etiology; in the United States (USA), previously published epidemiologic studies of ALS are limited and have been primarily confined to mortality data (1, 2). Previously, we have described a town in New Hampshire (NH) with 25 times the expected incidence rate of ALS (3). This study aims to describe the incidence and mortality trends of ALS in the state of NH, USA, over a four-year period, and to describe the demographics of this ALS population.

Methods: Records from regional ALS centers, clinics, and ALS organizations were retrospectively searched to identify patients diagnosed with ALS in NH from January 2004 to December 2007. Medical records were reviewed when possible to confirm diagnosis and extract details on demographics, tobacco use, and occupational history. Mortality data for 2004–2007 was obtained by reviewing state death certificates to identify those listing the ICD-10 code G12.2 for motor neuron disease as primary or contributing cause of death. Age-standardized incidence and mortality rates were calculated using US census data (4).

Results: We identified 118 NH residents diagnosed with ALS in 2004–2007. The age-standardized incidence rate per 100,000 population, ranged from 1.6 in 2004 to 2.5 in 2006. During the same 4-year period, the standardized mortality rate per 100,000 population varied from 3.5 in 2004 to 2.6 in 2007. Familial ALS was reported in 6%, and five cases were thought to have primary lateral sclerosis (PLS). ALS was more common among men (ratio 1.3:1). Men were more likely than women to have an earlier age of onset (59 ± 13.9 years vs. 65 ± 12.2 years, p = 0.01), and were 1.6 times more likely to have held a high-risk occupations, including medical, factory worker (machinist), carpentry (construction), and chemical production.

Conclusions: While localized areas with high incidence rates in NH have been previously reported, incidence and mortality rates of ALS in NH are overall similar to other industrialized nations (6). A prospective ALS registry will enable us to understand incidence rates and to identify environmental or occupational exposures related to earlier age of onset in males, and clarify the previously reported geographic disparity in ALS incidence.

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P139   INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN RHINELAND-PALATINATE, GERMANY – THE ALS REGISTRY RHINELAND–PALATINATE

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Background: In contrast to other Western countries (eg Ireland (1), Scotland (2), and Italy (3–5)) there is a lack of prospective and population-based epidemiological data on amyotrophic lateral sclerosis (ALS) in Germany.

Objectives: We intended to determine the incidence, course and phenotypic variety of ALS in Rhineland–Palatinate, a county in South-West Germany with about 4 million inhabitants.

Methods: During the period 2010–2011, consecutive patients with a first diagnosis of ALS according to the revised El Escorial criteria and were registered using multiple overlapping sources of case ascertainment. To assess the course of the disease patients were followed up in 3–6 months intervals. Death certificates from local health authorities served as an additional registration tool for deceased patients. Age-specific and age-standardised incidence rates were calculated to compare our results with results of other prospective registries.

Results: During the period 2010–2011, 146 incident patients were enrolled. The annual crude incidence for ALS in Rhineland–Palatinate was 1.8/100,000 person years (95% CI: 1.6–2.2). Male to female ratio was 1.1:1. Incidence increased with age reaching a peak in the 70–74 age group (10.3/100000 person years, 95% CI: 7.6–13.6) and declined thereafter. Mean age at diagnosis was 66.2 ($\pm$10.6) years. About 32% of patients presented with bulbar onset, 34% with symptoms in lower limbs, and 23% with symptom onset in upper limbs. 4% of patients had an initial trunk or respiratory involvement. Mortality rate within the first year of diagnosis was 26%.

Discussion and conclusion: The ALS registry Rhineland–Palatinate is the first population-based prospective ALS register in Germany. Incidence, phenotypic variety and one-year-mortality rate of ALS in Rhineland–Palatinate is within the range of previous prospective population-based registries in Western countries. Gender ratio is nearly balanced, which might reflect a better case ascertainment of female ALS patients.

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P140 CLUSTERING OF ALS IN FRANCE: RESULTS OF THE BMAALS STUDY

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Keywords: cluster, L-BMAA, France

Background: The BMAALS study aims to improve scientific knowledge about the possible link between ALS and cyanotoxin L-BMAA. We report here the results of the geoepidemiological step of this survey in identifying all incident cases on the area under consideration and searching for clusters.

Objective: To identify the presence of clusters of ALS among 10 French departments of France (58 254 km[2]) covering 5.2 million people followed for 9 years.

Methods: Multiple sources of information were used to ensure complete case ascertainment of individuals diagnosed with ALS between 2003 and 2011 according to Airlie House criteria in the following areas of France (i) Limousin region (Corrèze, Creuse, and Haute-Vienne departments); (ii) Rhone–Alpes region (Isere, Savoie, Haute Savoie, Drôme, and Ardèche departments); (iii) Languedoc Roussillon region (Pyrenees Orientales and Herault departments). Multiple sources of case were (i) French national ALS centers; (ii) hospitals and clinics; (iii) health insurance structures; and (iv) private neurologists. Crude incidence per 100,000 inhabitants was assessed using estimates of population from the Insee. Previously applied methods were used for cluster detection (1): Standardized Incidence Ratios (SIR) calculation used the overall incidence in the area as the reference. I Moran and Kulldorf statistics were also performed.

Results: Between 1 January 2003 and 31 December 2011, 1211 patients were diagnosed as having probable, probable laboratory supported or definite ALS in the area under study. The average annual crude incidence rate for those cases was 2.57 (95% CI: 2.43–2.71) per 100,000 person year of follow-up (PYFU). Standardized on 2010 European population the incidence was 2.50 (95% CI: 2.37–2.65) per 100,000

PYFU. Thirteen clusters of ALS were identified within the area. The median SIR value was 3.3 (95% CI: 1.2–7.1).

Discussion and conclusion: We report here the widest study of ALS incidence and clustering ever conducted in France using a thorough methodology. We based our calculation on the incidence assessed in a wide area of France covering 5.2 million inhabitants. The clusters that were identified will be explored for their relation with L-BMAA exposure during the next steps of the BMAALS study.

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P141 RESULTS FROM THE FIRST FRENCH ALS REGISTER: THE LIMOUSIN ALS REGISTER

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Keywords: register, incidence, France

Background: In France, until recently, there was no register of ALS cases. Hence description of the disease in France was only hospital based and then exposed to bias.

Objective: To assess the incidence of ALS in the Limousin region of France using multiple sources of cases ascertainment, and to describe the sociodemographical and clinical profile of new ALS cases.

Methods: A register has been settled in the Limousin region of France, 743,000 inhabitants (1). Multiple sources of information were used to ensure complete case ascertainment of individuals diagnosed with ALS according to Airlie House criteria, within the Limousin since 2000. Multiple sources of case were (i) Limousin ALS expert center and other ALS centers of France; (ii) private hospitals and clinics; (iii) health insurance structures (reimbursement of Rilutek® and long duration affection notification); and (iv) private neurologists. Data were collected in a secured database. Crude incidence per 100,000 inhabitants was assessed using estimates of population from the Insee (2). Direct standardized incidence based on 2010 US population was also calculated (3). Ninety-five percent confidence intervals (95% CI) were calculated assuming a poisson distribution.

Results: Between 1 January 2000 and 31 December 2011 (12 years), 279 patients were diagnosed as having possible,

probable, probable laboratory supported, or definite ALS. The median number of sources by patient was 2 (Interquartile range: 2–3). The average annual crude incidence rate was 3.18 per 100,000 person-years (95% CI 2.81–3.56). Standardized incidence on US population was 2.18 (95% CI 1.92–2.45). Mean age at diagnosis was 69.1 ± 11.2 years and the male/female sex-ratio was 1.45. First symptoms were mostly spinal (65.5%) and 6.45% of cases had familial ALS.

Discussion and conclusion: We report here the results of the first ALS register settled in France. The epidemiological profile of ALS incidence in Limousin, France is consistent with those published by other European registers (2). The register will be the base of analytic and geoepidemioloical studies (clustering). We are now extending the register in other region of France: Languedoc Roussillon and some departments of Rhône Alpes.

Acknowledgments: Sources of cases: French National coordination of ALS centers; Health insurance structures: Régime Général, Mutuelle Sociale Agricole, Régime Social des Indépendants, Caisse Nationale Militaire de Sécurité Sociale; Private Neurologists; Hospital and Clinics.

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P142   CLINICAL SPECTRUM AND NATURAL HISTORY OF MOTOR NEURON DISEASE IN KOREAN

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Keywords: ethnicity, motor neuron disease, survival

Background: The clinical spectrum of motor neuron disease (MND) is wide with a variable course. Recent studies in amyotrophic lateral sclerosis (ALS) suggest that ethnic difference may influence clinical and genetic characteristics of MND.

Objectives: We described the clinical profile and survival of Koreans with MND seen in a tertiary referral center in Seoul, Korea.

Methods: We analyzed clinical features of patients with 792 MND from our ALS Center and verified survival by telephone survey.

Results: ALS was diagnosis in 621 (78.4%) patients, PMA in 106 (13.3%), and Kennedy`s disease in 32 (4%). In ALS, onset of age were more likely to be lower (53.5- vs. 56.4-year-old), and individuals lived longer (39.9 vs. 31.5 months) than progressive muscular atrophy (PMA).

Discussion and conclusions: Although ALS and PMA had similar clinical characteristics, longer survival in ALS is a different finding to previous western studies.

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P143   TRANSITIONAL METAL CONTENTS IN SCALP HAIR AND LIFESTYLE OF ALS PATIENTS AND RESIDENTS IN THE KII PENINSULA, JAPAN: THE SECOND REPORT

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Keywords: neutron activation analysis, transitional metals, lifestyle

Background: A high incidence of amyotrophic lateral sclerosis (ALS) has continued in the Koza/Kozagawa/Kushimoto (K) area in the Kii Peninsula of Japan. We previously reported an elevation of urinary 8-hydroxydeoxyguanosine (8-OHdG), an oxidative stress marker, in patients with ALS in this area (K-ALS). Lifestyle might play a role in increasing oxidative stress.

Objective: The aim of this study is to investigate relationships between the contents of transitional metals in the scalp hair and lifestyle of the residents in this area (K-residents) and patients with K-ALS.

Methods: Hair samples were collected from patients with K-ALS, sporadic ALS, K-residents and controls and approximately 30 mg of each sample was subjected to neutron activation analysis at Kyoto University Research Reactor according to the protocol previously reported (1). As comparative standards, a human hair standard (NIES CRM No.13) and elemental standards were used. K-residents were asked to answer dichotomous questionnaires about lifestyle. The relationship between metal contents in the hair and the questionnaire data was analyzed statistically.

Results: Hair samples from seven patients with K-ALS, 10 patients with sporadic ALS, 86 K-residents and 19 controls were collected between 2010 and 2012 and the contents of Ca, Al, Cu, Mn and V were analyzed. The samples were not treated with chemical procedures including perm. The contents of V (148.3 ± 277.4 ppb, mean $\pm$ S.D.) and Mn (0.75 ± 0.66 ppm) of patients with K-ALS were higher than those of the controls (19.2 ± 11.1 and 0.22 ± 0.35, respectively, $p < 0.05$). Some of the K-residents showed high V in the hair; however, the mean content (28.6 ± 27.9 ppb) was not significantly different from the controls. The contents of V in the hair were positively correlated with the contents of Mn and Al in the hair. No correlation was found between the contents of these metals in the hair and urinary 8-OHdG in K-residents. K-residents who answered that they ate Japanese pickled vegetables daily showed higher hair V and Mn ($p < 0.05$ and $p = 0.056$, respectively) and who answered that they worked on a farm almost everyday showed higher Mn and Al in the hair than those who rarely did ($p < 0.01$, respectively).

Discussion and conclusion: The present results indicate that the contents of V and Mn in the scalp hair of patients with K-ALS were elevated, and it may be related to the lifestyle including eating habits and field labor in this area. These transitional metals may play a role in increasing oxidative stress on patients with K-ALS.

Reference:

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P144   PRELIMINARY REPORT OF AFRICAN CASES IN THE TROPALS STUDY – A SURVEY OF AMYOTROPHIC LATERAL SCLEROSIS IN TROPICAL AREAS

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Keywords: Africa, ethnic groups, tropics

Background: Epidemiological studies of amyotrophic lateral sclerosis (ALS) in the tropics are rare and their methodologies are heterogeneous (1). Many questions arise as regards the characteristics of this disease in the tropics.

Objective: To describe sociodemographical and clinical characteristics of ALS patients diagnosed in Tropical areas.

Methods: TROPALS (http://www.tropals.unilim.fr/) is a multicentre observational cohort study. A shared methodology and an online database allow centres to collect data in a standardized and homogeneous way. We will here focus on African cases only.

Results: Since May 2012, 40 African patients have been included in five centres (Benin, Mauritania, Senegal, Togo, and Tunisia), other centres are open (Burkina Faso, Gabon, and Mali) and six others are about to open. Mean age at diagnosis was 51.9 ± 13.5 years (two cases less than 25 years), male/female sex ratio was 2.4.

First symptoms were mostly spinal (72.5%) and 80.0% of patients (n = 32/40) had electroneuromyography for diagnosis purpose. At this time mean ALSFRS-R was 32.1 ± 10.5 and 75.0% of patients presented atypical symptoms (mostly dysautonomic or sphincter troubles). After diagnosis, 97.4% of patients were prescribed an occidental treatment: Rilutek® (n = 12), physiotherapy (n = 17), or symptomatic treatment (n = 12). 21.6% of patients used a traditional treatment based on infusion-decoction for two of them and of unknown type for six patients.

Discussion and conclusion: More inclusions are needed to produce precise estimations. Follow-up data are currently being collected. Our aim is to extend Tropals to other countries in Africa and other Tropical zones. Tropals study will allow us to improve the description of ALS characteristics, prognosis for patients, and comprehension of the disease under the tropics

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P145   REAPPRAISAL OF THE NOSOLOGICAL SIGNIFICANCE OF ALS-PDC MIXED CASES ON GUAM

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Keywords: nosological significance, ALS-PDC mixed case, Guam

Background: The incidence of amyotrophic lateral sclerosis (ALS) on Guam has greatly declined since 1970 and disappeared for these years. However, parkinsonism–dementia complex (PDC) has still retained a relatively high occurrence with a trend changing to late-onset dementia without parkinsonism (Marianas Dementia) (1). Recently, we have observed a similar trend in another ALS/PDC focus in the Kii Peninsula of Japan.

Objective: The purpose of this study is to examine whether these diseases are considered as a single entity with a spectrum of expressions: pure ALS and PDC at each end, and to clarify a nosological significance of the ALS-PDC mixed case.

Methods: Statistical analysis was carried out using the entire NINCDS files for over 25 years from 1958 to 1982 (2), including 540 cases in total; 243 ALS, 286 PDC and 29 mixed cases. Fisher's exact test was used to examine the independence of occurrence between ALS and PDC, among the groups of year at onset divided by the 5-year study periods and those of age at onset divided by decades. The strength of association between them was evaluated by Odds ratio. We used the population in 1970 as the standard base-population for analysis, at the mid-point of the whole study period.

Results: In the last 5-year period of 1978–1982, the incidence rates of both ALS and PDC cases markedly decreased below one-fifths of that in the first 5-year period of 1958–1962. On the contrary, the incidence rate of ALS–PDC mixed cases kept constant throughout the study period. The independence of occurrence between ALS and PDC cases was significantly denied among any groups of year at onset divided by the 5-year period and those of age at onset divided by the decades (Fisher's exact test, $p < 0.0001$). Odds ratios for 20 years from 1958 to 1977 had gradually increased and sharply rose up during the last 5-year period.

Discussion: These results suggested that the ALS-PDC mixed cases might be a core disease rather than a by-chance, combined form between ALS and PDC, and not influenced merely by rapid socioeconomic changes on Guam. We will also discuss a similar trend of ALS/PDC in the Kii Peninsula of Japan.

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P146   ALSFRS-R DECLINE IN PATIENTS FROM THE EMILIA-ROMAGNA REGISTER FOR ALS (ERRALS)

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Keywords: ALSFRS-R, population based study, disease progression

Background: The ALSFRS-R is an attractive primary outcome measure in clinical trials of ALS because it is validated, easy to administer, minimizes dropout, reduces cost, and correlates with survival. The ALSFRS-R is also a measure of global function. Few studies, mainly based on ALS centers data, examined the topic of ALSFRS-R decline during the disease course, and the relationship between clinical factors and rate of decline of the scale.

We report the results from a prospective population-based epidemiological study in Emilia Romagna Region, Italy, to describe the rate of decline of ALSFRS-R in relation to the clinical features and phenotypes of ALS patients from 2009.

Methods: This study was performed innine provinces and 11 local health units of Emilia Romagna (population 4.4 million inhabitants), with the involvement of 17 neurological departments. From 2009 onwards, a prospective registry has been collecting all cases of incident ALS among residents in Emilia Romagna region. For each patient, the main demographic and clinical information were collected by the caring physicians. In addition, a case report form has been completed during each patient follow-up.

Results: In the period from 1 January 2009 to 31 December 2011 in Emilia Romagna, 344 patients received a new diagnosis of ALS. Mean time from onset to diagnosis was 12.4 months. The follow-up period ranged from 12 to 48 months. Mean ALSFRS-R score at diagnosis was 39.7 (M = 41.3 and F = 37.8); mean rate of decline in the first year was 0.8 points/month (M 1.03, F 0.55), versus 0.3 points/month during the second year (M = 0.27, F = 0.27). The rate of decline was influenced by age, site of onset and disease phenotype, mainly during the first year after the diagnosis: the rate of decline was 1.08 points/months for p... ...

>70 yrs of age at onset, compared to 0.69 for patients having <71 yrs. Bulbar onset patients lost 1.15 points/month whereas spinal onset patients only 0.60 points/month. Patients with bulbar phenotype lost 1.10 points/months whereas patients with other clinical phenotype (classic, flail, and UMNp) lost 0.7 points/month to ALSFRS-R. Conversely, spinal onset patients had a major decline in FVC than bulbar patients, the latter starting with lower FVC scores at diagnosis.

Discussion and conclusion: Higher rate of decline in ALSFRS-R is present in older age-at-onset and bulbar patients. The first year of the illness after diagnosis shows the most rapid rates of decline, especially for male and bulbar patients. These data can be useful for allocation of patients within clinical trials and for managing the care of ALS patients.

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P147   CLINICAL CHARACTERISTICS OF PATIENTS WITH YOUNG-ONSET ALS

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Keywords: clinical, prognosis, young onset ALS

Background: Young-onset amyotrophic lateral sclerosis (ALS) is sporadic ALS typically involving patients less than 45 years. Early onset ALS accounts for approximately 10% of sporadic ALS cases. No one knows the time elapsed since the onset of neurodegeneration and the appearance of symptoms. Phenotypic forms are very variable, being very rare bulbar involvement. Although disease progression is highly variable among all reported cases, there is tendency in slowly progressive symmetric weakness.

Objectives: To describe the clinical characteristics and survival analysis of patients with early onset ALS.

Methods: This is a retrospective study of 43 patients diagnosed with young-onset ALS under the age of 45 years analyzing clinical, prognostic factors and survival, and comparing these data with 180 adult patients with ALS attended in the University Hospital of Bellvitge in the province of Barcelona from the period 1990–2013.

We perform comparative analysis using Chi-square tests. Significant p-values less than 0.05 (significance level 5% alpha). The estimate of survival was performed using Kaplan Meier Test and the Log-Rank Test.

Results: 43 patients were diagnosed with early-onset ALS, 26% of the entire series. Two patients had history of Frontotemporal Dementia. The male female ratio was 2:1. Spinal onset was observed in 93%. The overall survival was 57 months (older than 45 years, 31 months), 43 months for spinal and 26 months for bulbar onset. The analysis of the different variables that influence survival showed significant differences only in ventilated patients (p = 0.018).

Conclusions: Young patients represent 26% of the entire series. Being young is an independent predictor of increased survival and represents a distinct clinical variant predominantly spinal form, with a higher prevalence in men and increased survival compared to adults. The bulbar form is rare, regardless of gender. Noninvasive ventilation played a significant increase in survival. This study found no benefit

in the treatment with riluzole or with the use of enteral nutrition.

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P148   RARE ALS VARIANTS IN THE TURKISH POPULATION

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Keywords: SETX gene, VAPB gene, essential tremor

Backround: Mutations in SETX, SOD1, and VAPB genes have been identified in amyotrophıc lateral sclerosis (ALS).

Objectives: Juvenile ALS amyotrophic lateral sclerosis (ALS) is a form of chronic motor neuron disease characterized by combined upper and lower motor neuron symptoms and signs, with onset prior to age 25 years.

ALS4 is an autosomal dominant form of juvenile onset ALS associated with slow progression, severe muscle weakness and pyramidal signs, in the absence of bulbar and sensory abnormalities. We studied two ALS patients with novel mutations. One had juvenile onset of ALS. The second case resembled VAPB (ALS8). The vesicle-associated membrane protein/synaptobrevin-associated membrane protein B (VAPB) Pro56Ser mutation has been identified in Brazillian families showing various motor neuron syndromes.

Case report:

- Case Study 1, a 25-year-old female presented in December 2010 with an ongoing weakness in her left arm lasting 5–6 months. The patient demonstrated weight loss and respiratory distress and has a family history of consanguinity. Other muscles of extremities were normal. Babinsky sign was positive. Bilaterally Jaw reflex was positive. The patient demonstrated bulbar signs and anterior horn finding were determined by EMG examination. Following neurogenetic examinatıon, SOD1 mutation was negative but the homozygote nonconserved missense variant 682E in SETX gene was positive. Riluzole was started as treatment, dosage is 100mg/per day. FVC is 60% in respiratory test.
- Case Study 2, a 77-year-old male patient with diabetes mellitus disease for 30 years presented to our clinic in August 2008 with a tremor in his hands. The tremor in his right hand had occurred since 1998. The patient has a family history of consanguinity. Two-sided tremor and the proof of cogwheel were determined in the neurological examination and PD was diagnosed. There were no further PD characteristics except minimal tremor, the UPRS was 5 and cranial MR examination was normal. Genetic research was carried out on patients due to intermarriage. Following neurogenetic investigation, the homozygous, non-conserved, missense variant M93T in VAPB gene ALS 8 was identified.

Conclusıons: This study identifies new genetic associations with ALS and provides phenotype correlations with both previously reported and novel mutations. In this study, we

wanted to report these very occasional cases which have clinical characteristics and genetic finding.

Reference:

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P149   UBQLN2 IN A JUVENILE ALS WITHOUT DEMENTIA

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Keywords: juvenile ALS, dementia,UBQLN2

Background: Amyotrophic lateral sclerosis (ALS) is a paralytic and usually fatal disorder caused by motor-neuron degeneration in the brain and spinal cord. Most cases of ALS are sporadic but about 5–10% are familial. Mutations in superoxide dismutase 1(SOD1),TAR DNA-binding protein(TARDBP, also known as TDP43)and fused in sarcoma(FUS, also known as translocated in liposarcoma, TLS) account for approximately 30% of classic familial ALS. Mutations in several other genes have also been reported as rare causes of ALS or ALS-like syndromes.

A case report: A 16-year-old male was examined with a weakness in his right leg which had occurred for 2 years, heel disturbance were determined. The patient demonstrated bilateral atrophy and fasiculation in both distal lower extremities as determined by a neurological examination. Two-sided dropped feet and tibialis anterior weakness were also found. Lumbar and thoracic MR examination was normal. Routine laboratory research was normal. EMG showed anterior horn findings. DNA sequence analyses found no SOD gene mutations however the M329L mutation was identified in 392 position of UBQLN2.

Conclusion: In this study, we report a juvenile-type case with ALS 15 without FTD diagnosis, with a mutation in UBQLN2. This case was discussed.

P150   PHYSICAL ACTIVITY: A RISK FACTOR FOR MOTOR NEURONE DISEASE?

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Keywords: physical activity, risk

Background: Reports suggesting a higher incidence of motor neurone disease (MND) in professional sportsmen have motivated research into a potential aetiological association between physical activity (PA) and MND, as part of a gene–environment interaction (1). Unrelated research demonstrates that PA normally augments some known MND

pathogenic mechanisms and may also upregulate angiogenic and neurotrophic genes associated with MND (2,3). However, definitive evidence is lacking, with methodological limitations to prior studies.

Objectives: To conduct a case-control study to determine any association of PA with the development of MND, using sound methodology.

Methods: Collaborating with Cambridge MRC Epidemiology Unit, we designed an interview-administered PA questionnaire (HAPAQ), which collects data regarding total adulthood PA (home, work, transport and leisure). Questionnaire validity was determined by comparing HAPAQ-derived data from 100 interviews with historical objective PA energy expenditure (PAEE) measurements from the same individuals. Subsequently, HAPAQ was used to interview incident sporadic MND cases, identified from regional MND services, and age- and gender-matched controls from general practice patient databases. Using the Compendium of Physical Activities (4), individual PAEE scores are determined from questionnaire-derived data. Case and control PAEE will be compared using conditional logistic regression, adjusting for potential confounders.

Results: HAPAQ-derived data correlated with objective total and vigorous PAEE measurements ($r = 0.44$, $p < 0.001$; $r = 0.40$, $p < 0.001$, respectively) (5). To date, 160 cases (63% male) and 300 controls (63% male) have been recruited (age range: 26–91yrs, mean age 64 years).

Discussion and conclusion: As one of the first questionnaires to be validated against historical objective PAEE measurements, HAPAQ accurately ranks individuals by adulthood PA using a standardised data collection method. Additional strengths of the study include a population-based recruitment approach, El-Escorial defined cases, blinding to the research hypothesis and confounder adjustment. Discussion of results analysis will be submitted as an updated abstract.

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P151   AMYOTROPHIC LATERAL SCLEROSIS, PHYSICAL ACTIVITY AND SPORT: A LITERATURE REVIEW

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Keywords: physical activity, sport, soccer

Background: Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease of unknown aetiology. Occupational and environmental exposures may contribute to the risk of developing ALS. Several hypotheses have been proposed to explain a relationship between the occurrence of ALS and a number of exogenous risk factors such as physical activity (PA), trauma, and exposure to toxic substances alone or in combination with predisposing genetic characteristics.

Objective: To elucidate whether physical activity and sport increase the risk of developing ALS.

Methods: To summarize the available evidence and to conclude on the association between ALS, sport and PA, a systematic review of the epidemiological literature on PA as a potential determinant of ALS was performed according to the MOOSE guidelines. From PubMed (MEDLINE), SCOPUS, Science Direct (Elsevier), Ingenta Connect, Refdoc (the INIST/CNRS), and the Cochrane databases up to January 2013, selected studies were methodologically appraised according to Armon's classification system for ALS risk factor studies (1). References of previous meta-analyses were also considered and experts were contacted to identify relevant unpublished studies. The search was not limited in time but to articles in French and English languages.

Results: Of 464 potentially relevant studies, only 36 studies were eligible. Of these, 12 were cohort studies (mostly descriptive), 20 case-control studies and four case-series studies. Of all, one study fell into Armon's level of evidence class I *(unpublished)*: five as class II; eight studies were classified as class III; 16 as class IV and six as class V. Synthesis of data was stratified by definition of PA which strongly varied across studies: (i) sport and PA; (ii) occupational activity (farmers, foresters, fishermen and mason); (iii) soccer; (iv) American football; and (v) proxies. As regards PA and sport, the review achieves a level B of evidence, meaning that it is probably not a risk factor. Nevertheless this result was not stable when the unpublished work was not included.

Conclusion: PA appears to be probably not a risk factor for ALS. However, some data suggest an accelerating effect of PA in predisposed individuals. Further good-quality studies (class I or II) are needed to stabilize and confirm these results.

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1. Armon C. An evidence-based medicine approach to the evaluation of the role of exogenous risk factors in sporadic amyotrophic lateral sclerosis. Neuroepidemiology 2003; 22(4):217–228.

P152   THE IMPACT OF PHYSICAL IMPAIRMENT ON EMOTIONAL FUNCTIONING IN ALS SEEN THROUGH THE PATIENT'S EYE

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Keywords: emotional functioning, physical impairment, patient-reported outcome measure

Background: The diagnosis of amyotrophic lateral sclerosis (ALS) and its relentless progression are a permanent emotional burden for these patients. However, according to the literature emotional functioning does not seem to correlate with physical impairment (1).

Objective: To investigate the impact of physical impairment on emotional functioning when consistently measured by patient-reported health assessment instruments.

Methods: Eighty-five consecutive patients were asked to participate in the study. Emotional functioning was assessed by the ALSAQ-40, a validated patient-reported disease specific tool to assess quality of life. Physical impairment was evaluated by the recently validated extended ALSFRS (ALSFRS-EX) as a patient-reported outcome measure, comprising three additional items to increase sensitivity of the original ALSFRS-R for severe functional impairment (2,3). Pairwise Spearman rank correlation coefficients were used to correlate the ALSFRS-EX with the subscale "emotional functioning" of the ALSAQ-40.

Results: Seventy-six patients participated in the study. We noticed significant negative correlations between emotional functioning and the ALSFRS-EX items 'facial expression'; 'dressing'; 'turning in bed'; 'walking';'getting around at home' ($p < 0.01$) and 'swallowing'; 'handwriting'; 'feeding'; 'finger movement'; 'climbing stairs'; and 'breathing on back' ($p < 0.05$).

The items of the emotional functioning subscale 'being bored'; 'feeling as if I have no freedom' ; and 'worried to be a burden' were significantly correlated with gross and fine motor function impairment ($p < 0.01$), 'feeling lonely' and 'feeling hopeless' were correlated with bulbar, gross and fine motor impairment ($p < 0.05$). Bulbar involvement was significantly correlated with 'feeling embarrassed in social situations'; 'feeling worried about future' ($p < 0.01$); 'feeling depressed'; and 'feeling angry' ($p < 0.05$). Fine motor impairment was also correlated with 'feeling worried about future' ($p < 0.01$).

Discussion: Up to now the influence of physical impairment on emotional functioning was found to be only minor or not even present (1). Patient-reported assessment may be more suitable to capture relevant and plausible correlations more sensitively. Our results indicate how multifaceted the impact of physical decline on emotional functioning is when assessed by patient-completed health assessment instruments, thereby directly seen through the patient's eye.

Conclusion: The impact of physical impairment on emotional functioning should not be underestimated. Timely and

comprehensive supportive therapy is crucial to improve emotional functioning as an important domain of quality of life in patients with ALS.

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P153   NECK WEAKNESS IS A POTENT PROGNOSTIC FACTOR IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: neck weakness, prognostic factor, cohort study

Background: Muscle weakness in particular regions of the body affect the prognosis of ALS, although it has not been sufficiently determined which regions are most predictive.

Objectives: To clarify the emergence of muscle weakness in regions of the body that affect survival and deterioration in activities of daily living (ADLs) in ALS patients.

Methods: We conducted a multi-center-based prospective cohort study of ALS patients. We enrolled 401 sporadic ALS patients. Death or the introduction of invasive ventilation was defined as the primary endpoint, and the time to five clinical markers of ADL deterioration associated with bulbar paralysis or limb weakness were defined as ADL milestones. Muscle weakness was assessed in the neck flexor muscles; the bilateral abductors of the shoulders; the bilateral wrist extensor muscles; the bilateral flexor muscles of the hips; and the bilateral ankle dorsiflexion muscles. We

performed Cox proportional hazards regression analyses for the primary endpoint and the five ADL milestones, adjusting for known covariate prognostic factors for ALS, and compared survival curves using the Kaplan–Meier method and a log-rank test.

Results: The MRC score for the neck flexors was the most significant prognostic factor for the primary endpoint (HR: 0.74, p < 0.001), loss of speech (HR: 0.66, p < 0.001), and loss of swallowing function (HR: 0.73, p < 0.001), and was one of the significant prognostic factors for loss of upper limb function, difficulty turning in bed, and loss of walking ability (HR: 0.77, p = 0.001; HR: 0.77, p = 0.002; and HR: 0.80, p = 0.008, respectively). The MRC score for the neck flexors was also a significant prognostic factor for covariates of the previously reported prognostic factors. We divided the patients into four categories according to their MRC score for the neck flexors (ie 5, 4, 3, and ≤ 2). All of the differences among the Kaplan–Meier curves for the primary endpoint and each ADL milestone were significant according to a log-rank test (p < 0.001).

Discussion and conclusion: The neck flexor muscles are mainly innervated by motor neurons in the cervical cord (C1–8) and accessory nerve nuclei. Motor neurons for the neck flexion muscles are contiguous or overlapping with those for the respiratory muscles, the bulbar muscles, and the upper limb muscles. It may be speculated that if the contiguous spreading of motor neuron degeneration occurs according to the local spreading hypothesis, neck flexion impairment may eventually affect survival and deterioration in ADLs. In conclusion, neck weakness is an independent prognostic factor for survival and deterioration in ADLs in ALS patients.

P154   OCCUPATIONAL EXPOSURE TO ELECTRIC SHOCKS AND MAGNETIC FIELDS AND MORTALITY DUE TO MOTOR NEURON DISEASE

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Keywords: occupational exposures, electric shocks, magnetic fields

Background: Motor neuron disease (MND) is consistently linked to electrical occupations, defined as occupations with typically higher exposure to magnetic fields (MF), in epidemiologic studies. However, the association with measured MF levels is weaker (1). Exposure to electric shocks (ES) is proposed as an explanation for the observed association between electrical occupations and MND (2). To date, no epidemiologic study has evaluated the relationship between ES and MND.

Objectives: To examine the association between occupational exposure to ES and MF, and MND, in a case-control study relying on U.S. mortality data between 1991 and 1999.

Methods: For each of the 5886 MND deaths, 10 controls were selected from other deaths and matched on sex, age, year, and region. Occupations obtained from death certificates were linked to job exposure matrices for ES and MF. Mortality odds ratios (MOR) were calculated using conditional

logistic regression, adjusting for education level, race, and ethnicity included in models.

Results: For ES, MND MORs were 0.73 (95% confidence intervals (CI): 0.67–0.79) for high exposure and 0.90 (95% CI: 0.84–0.97) for medium exposure compared to low. For MF, MND MORs were 1.09 (95% CI: 1.00–1.19) for high exposure and 1.09 (95% CI: 0.96–1.23) for medium exposure compared to low. For electrical occupations, MND MOR was 1.23 (95% CI: 1.04, 1.47) compared to non-electrical occupations. MND mortality was increased for electric occupations within medium- and low-exposure categories of both ES and MF, but not within high exposure to MF or ES.

Discussion: Among U.S. deaths, we found an inverse association between occupational ES and MND and no consistent association between MF exposure and MND. Similar to others, we observed an increased risk for MND for electrical occupations. However, exposure to ES and MF did not account for this increased risk. We present the first effort to separate the effects of two correlated exposures within electrical occupations in the U.S. population.

Conclusions: Current results are in support of an association between electrical occupations and MND, but provide no evidence that the association is explained by occupational exposure to ES or MF.

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THEME 7 GENETICS

THEME 7 GENETICS

P155 GENETIC COUNSELLING IN ALS: FACTS AND UNCERTAINTIES

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Keywords: genetic counselling

Background: The clinical approach to patients with amyotrophic lateral sclerosis (ALS) has been largely modified by the identification of several novel genes, detection of gene mutations in apparently sporadic patients, and discovery of the strict genetic and clinical relation of ALS with frontotemporal dementia (FTD). Consequently, clinicians are increasingly called to provide genetic testing and counselling both for ALS patients and their relatives.

Methods: A review of the existing literature on the genetics of ALS, and of other neurological late-onset, life-threatening disorders, and cancer will be presented and critically analysed.

Results: Following the Consensus meeting in Corteranzo, Italy, of a group of neurologists, geneticists, psychologists and ethicist with specific interest in genetics of ALS, detailed clinical suggestions were drafted and are now in press. We will present and discuss the consensus recommendations to enable neurologists and ALS specialists to provide optimal multidisciplinary clinical and genetic counselling to patients and families.

Genetic testing should be offered to ALS patients with a first- or second-degree relative with ALS, FTD or both, and it should be discussed with, but not offered to, all other ALS patients, with special emphasis on its major uncertainties in apparently sporadic ALS patients. The presence of co-morbid FTD in ALS patients should be assessed in order to establish their decision-making capacity, especially when signing informed consent for future genetic studies. Presently, genetic testing should not be proposed to asymptomatic at risk subjects, unless they specifically request it and/or are enrolled in research programs. We discourage parents from requiring ALS genetic analysis for their children and recommend that they be explained that genetic testing removes the child's ability to make an informed decision about testing when they reach adulthood and it carries major potential psychosocial implications.

Genetic counselling in ALS should take into account (1) uncertainties about the pathogenicity and penetrance of some genetic mutations; (2) possible presence of mutations of different genes in the same individual; (3) poor genotypic and phenotypic correlation in most ALS genes; and (4) phenotypic pleiotropy of some genes. Though psychological, social, ethical and legal implications of genetic testing are still relatively unexplored in ALS, a wealth of empiric data is available for other diseases such as cancer. We therefore also recommend in ALS a multidisciplinary counselling addressing all relevant medical, psychological and social issues, including sharing tests results to family members and risk for genetic discrimination. The ramifications of genetic testing on biological relatives should be made clear before any subject is tested. The meaning and value of the 'Right Not to Know' of both patients and family members should be familiar to all clinicians and discussed with all patients choosing to undergo genetic testing, and during the decision-making process regarding disclosure of their genetic testing results.

P156 WHOLE BLOOD GENE EXPRESSION PROFILES DISCRIMINATE ALS PATIENTS FROM HEALTHY CONTROLS

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Keywords: biomarker, gene-expression, micro-array

Introduction: Amyotrophic lateral sclerosis is often diagnosed per exclusionem. Although median survival after disease onset is 3 years, there is a considerable inter-individual variance. Biomarkers that provide diagnostic or prognostic information, and that can be used to monitor disease progression in clinical trials are highly needed in this disease (1). In the field of oncology, gene-expression profiling of tumor tissues has proven to yield valuable information for tumor classification, treatment options and prognosis (2).

Methods: We used whole blood gene expression profiling in order to try to identify a gene expression profile that is characteristic for ALS. Included were 397 ALS patients and 645 control subjects divided into a training set, test set and an independent validation set to ensure robustness of results. Whole blood messenger RNA was hybridized to Illumina HumantHT-12 v3 and v4 BeadChips. Data were corrected applying surrogate variable analysis. The nearest shrunken centroid algorithm was used to train, test and validate our model.

Results: We have identified a set of 106 genes that discriminated ALS patients from control subjects with high accuracy correctly classifying 89% of the samples in our test set and 80% in the validation set. Gene ontology analysis of gene functions revealed that the 106 genes were mainly involved in

RNA binding, an important process in motor neuron disease biology. Survival could not be predicted reliably.

Discussion: Clinical applicability will be further studied comparing ALS patients with patients suffering from a condition that mimics ALS. Furthermore longitudinal studies will be able to observe changes in expression of these genes to monitor disease progression.

Conclusion: We here show that whole blood gene expression profiles can be used to reliably discriminate ALS patients from controls.

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P157 CHANGES IN MICRO-RNAS' EXPRESSION DISCLOSE NOVEL LINKS BETWEEN ALS AND INFLAMMATION

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Keywords: microglia, microRNAs, P2X7 receptor

Background: Micro-RNAs regulate gene expression at the post-transcriptional level and are emerging as key modulators of the immune system whose dysfunction contributes to the progression of several neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS) (1). ALS is a non-cell-autonomous disease targeting both motor neurons and neighbouring glia, with microgliosis substantially contributing to neurodegeneration. While purinergic P2X7 receptor has a recognized role in neuroinflammation and ALS pathogenesis (2), its mechanistic signalling is only partially known. Despite several studies, which have identified a prominent and complex role of miRNAs as key modulators of signal propagation, there are only a few works on miRNAs regulation of purinergic systems and ALS (3).

Objectives: The aim of this work was to provide a map of those miRNAs that are differentially expressed in ALS, and to identify their implication in the pathogenesis. We examined primary microglia cultures from SOD1-G93A mice and human serum. We compared microRNAs transcriptional profiling in resting conditions and after activation of P2X7 receptor.

Results: We identified a strong upregulation of immune-enriched microRNAs transcriptome in ALS resting microglia and after P2X7 receptor stimulation, and recognized miR-22, miR-155, miR-125b and miR-146b as important microglia activators. In particular, by luciferase assays and lentiviral based microRNAs overexpression, we proved that miR-365 and miR-125b, suppressing the IL-6/STAT3 pathway, determine an increase of TNFalpha transcription. Furthermore, since TNFalpha upregulates miR-125b, we might recognize the induction of miR-365 and miR-125b as the gateway of a vicious cycle culminating in abnormal TNFalpha release. Finally, by analyzing circulating miRNAs levels in serum of sporadic ALS patients, we found a significant decrease of miR-195, miR-134 and miR-150, known to be involved in immune system functions and P2X7 receptor regulation.

Discussion and conclusion: These results strengthen the impact of microRNAs in modulating genes linked to inflammation and ALS. Moreover, they identify some specific micro-RNAs that might directly contribute to the outcome of ALS and might act as diagnostic markers or novel therapeutics for the disease.

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P158 HOMOZYGOSITY ANALYSIS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: genetics, homozygosity, recessive variant

Background: Amyotrophic lateral sclerosis (ALS) can be familial or sporadic, with dominant and recess

recognized in familial cases. Nevertheless, sporadic ALS may be caused by rare homozygous recessive mutations with no family history.

Objectives: To determine any excess of homozygosity in ALS cases and potential regions that may carry recessive disease-associated variants.

Methods: We studied a multinational pooled analysis of GWAS data in five ALS cohorts. PLINK v1.07 was used to determine the homozygous segments and any potential recessive variant leading to the disease. Association analysis was performed with PLINK algorithm with case control permutation.

Results: Two thousand and seventeen ALS cases and 6918 controls were studied in the pooled analysis. There were more regions of homozygosity segments per case ($p = 1 \times 10^{-5}$), a greater proportion of cases harboured homozygosity ($p = 2 \times 10^{-5}$), a longer average length of segment ($p = 1 \times 10^{-5}$), a longer total genome coverage ($p = 1 \times 10^{-5}$'), and a higher rate of these segments overlapped with RefSeq gene regions ($p = 1 \times 10^{-5}$), in ALS patients than controls. Positive associations were found in the chromosome 21 *SOD1* region, and also chromosome 1 2.9–4.8Mb.

Discussion and conclusion: The association in chromosome 21 *SOD1* region is partly contributed by over-representation of a group of *SOD1* D90A mutation carriers in one of the cohorts. A follow-up of the genes in chromosome 1 using next generation sequencing is currently on going.

P159   USING WHOLE GENOME SEQUENCING (WGS) TO SOLVE THE SPG27 QUESTION

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Background: Hereditary spastic paraplegia (HSP) is a motor neuron disease for which the key symptoms are lower limb spasticity and weakness because of progressive neurodegeneration events. The group of HSP is genetically and clinically heterogeneous with different modes of inheritance reported (dominant, recessive, and X-linked) and various levels of symptom complexity; pure and complicated forms have been described. To date, over 50 spastic paraplegia loci were identified and 30 causatives genes have been identified. A previous linkage analysis on an autosomal recessive pure HSP family identified a locus (*SPG27*) on the 10q22.1–10q24.1 region; a locus partially overlapping the *SPG9* locus.

Objective: To identify the causative gene, we performed a Whole Genome Sequencing (WGS) approach from Illumina, using their standard method. Three affected individuals were selected amongst the seven affected siblings. TruSeq kit was used as library and sequencing was done on HiSeq reaching a minimum of 30X coverage. Data analysis was performed using CASAVA (consensus sequence from ELAND alignment, called SNPs, and small indels).

Results: From a total of 68,994 variants shared by the three affected individuals, we filtered the variants list to keep only exonic variants that were not found in Exome Variant Server and also not found in dbSNP. We hand up with 23 rare variants to validate by traditional Sanger method.

Discussion: Finally, it will be possible to identify the causative gene by showing a perfect cosegregation with the clinical status of the whole family members.

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P160 ALS MOLECULAR GENETIC ANALYSIS: EXPERIENCE AT GENOA'S MEDICAL GENETICS UNIT

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Keywords: genetic analysis, mutation, expansion

Background: Research on genetic factors in ALS is rapidly advancing and recently new genes are recognized to be related to the disease. However, despite recent advances, most of the genes are still unknown. Considerable work is ongoing to discover such genes and elucidate specific therapy for mutation carriers.

Objective: Aim of the study was to review genetic features of a cohort of sporadic and familial ALS patients who underwent routine diagnostic genetics tests from 2000 to date, at the Medical Genetics Unit of the IRCCS AOU S. Martino-IST, Genova. Furthermore the analysis of candidate genes was performed.

Methods: After adequate information and signed written consent, 200 patients underwent DNA analysis for ALS. ALS clinical diagnosis was based on the revised El Escorial criteria. Family history was investigated, when possible, and demographic data and clinical characteristics of index cases were pointed out.
    Exons and splice junctions of *SOD1, TARDBP* (exon 6); *FUS-TLS* (exons 6 and 15); *FIG4,VCP* (exons: 2, 3, 5, 6, and 14); *OPTN* (exons: 3, 4, 9, 10, 12, and 14) genes were examined by direct nucleotide sequence analysis. Pathological expansions in *ATXN2* (CAG)n and *C9ORF72* (GGGGCC)n were also analysed by TP-PCR.

Results: Out of 200 patients included in the study, 19 (9.5%) carried a mutation in one of the genes considered. All mutations were detected in heterozygous condition. Mutations found were eight *SOD1* (42%), five in FALS and three in SALS patients; three *TARDBP* (15.8%), one FALS and the other SALS; two *FUS-TLS* (10.5%) in FALS patients; Pathological expansions in *C9ORF72* were found in six patients (31.6%) three FALS and three SALS; No mutations in *VCP, FIG4,* and *OPTN* genes were found in the analyzed exons; No expansions were found in the *ATXN2* gene.

Discussion: In our ALS series we found that 9.5% of patients carry a genetic mutation. In our population *SOD1* mutations and *C9ORF72* expansion were the most represented. The complexity of the *C9ORF72* expanded phenotype, which includes cases with ALS, ALS-FTD, and FTD, changes the current notion of familial ALS, requiring collection of family members with different and often complex phenotypes. Furthermore, the presence of cognitive impairment adds more issues related to informed consent for genetic studies.

Conclusions: The overall percentage of our mutated ALS cases (9.5%) is in agreement with that discussed in the literature. Geneticists are increasingly facing questions about newly identified genes and their role in the ALS pathogenesis, as well as penetrance of individual gene mutations, severity and modifier factors of phenotypes. Genetic counselling is a useful setting for managing such problems, according to shared new guidelines which have to be discussed and identified at a national and international level.

P161 IMPROVING THE KNOWLEDGE OF ALS GENETICS: NOVEL SOD1 VARIANTS AND RECESSIVE FUS MUTATION

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Background: Mutations in C9ORF72, SOD1, TARDBP and FUS genes are currently considered as the most common genetic cause of familial (FALS) and sporadic (SALS) forms of ALS.

Objective: The aim of the study was to estimate the mutation frequency of these genes in a large cohort of ALS patients referred to the ALS Clinic of the University, Padova. Moreover, considering the emerging clinical and genetic overlap between ALS and frontotemporal dementia (FTD), a second panel of FTD patients was also investigated for mutations on C9ORF72, FUS and TARDBP genes.

Methods: We investigated a total of 354 unrelated patients, 45 of them with reported family history (FALS) and 309 classified as sporadic/isolated cases (SALS). We performed Sanger sequencing of SOD1, TARDBP and FUS genes. In addition, a cohort of 73 FTD patients (25% with positive family history) was also evaluated for point mutations on FUS and TARDBP genes. The expanded GGGGCC hexanucleotide repeats in the C9ORF72 gene was genotyped in all ALS/FTD patients.

Results: We identified several pathogenic mutations in ALS patients: six in SOD1, two in TARDBP and two in FUS. All but two of these mutations were already reported by other groups. A new SOD1 mutation (p.Glu41Gly) was detected in a FALS patient. We also identified a new single base deletion in homozygous state (p.Gly496Glyfs*31) in the FUS gene: the mutation carrier reported no family history for ALS, suggesting a recessive inheritance of the disease. No point mutations were identified on FTD patient panel. The frequencies of expanded repeats in C9ORF72 were similar in ALS and FTD patients: 22% in the familiar cases and about 5% in the sporadic patients.

Discussion and conclusion: Our mutation screening confirms C9ORF72 as the main causative gene both in ALS and FTD patients. Moreover, this work highlights the importance of molecular testing of SOD1, TARDBP, and FUS in ALS patients identifying a new SOD1 mutation and pointing out the chance of an autosomal recessive pattern of FUS mutation inheritance. Finally, our results suggest that mutations in TARDBP and FUS genes are rare causes of FTD.

P162   CHARACTERIZING THE GENETIC HETEROGENEITY OF ALS THROUGH MASSIVELY PARALLEL TARGETED RESEQUENCING

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Keywords: genetic epidemiology, heterogeneity, oligogenic

Background: ALS is a highly heterogeneous disorder. A formal understanding of disease heterogeneity may greatly advance a variety of clinical and research efforts.

Objectives: (1) To describe the genetic heterogeneity of ALS in Ireland by characterizing the variation across 33 previously reported disease genes; (2) to compare the observed frequencies of disease variants with those reported by studies of representative patient cohorts from other European populations; and (3) to search for correlations in the occurrence of putative disease variants which may be indicative of oligogenic aetiology.

Methods: Four hundred and forty-four representative Irish ALS cases (50 fALS and 394 sALS) and 311 age and geographically matched controls were analyzed by multiplexed targeted high-throughput sequencing. Known and potential ALS variants were subsequently identified using an assortment of bioinformatic analyses.

Results: 17.1% of patients were found to carry known or potential high penetrance ALS variants. The frequency of Mendelian disease gene variants was 12.8% (*C9orf72* 8.78%; *SETX* 2 48%; *ALS2* 1.58%; *FUS* 0.45%; *TARDBP* 0.45%; *OPTN* 0.23%; *VCP* 0.23%; *ANG, SOD1, VAPB* 0%), while the frequency of low penetrance/tentative ALS gene variants was 4.7%. 9.7% of patients (30% of fALS and 7.1% of sALS) carried previously described ALS variants (*C9orf72* 8.78%; *FUS* 0.45%; *TARDBP* 0.45%). Only 1.58% of patients were found to carry multiple putative disease variants, yet this included all identified carriers of one established ALS variant (n = 2/2 sALS, p < 0.01). Comparison of our results with those from studies of other representative European cohorts revealed significant differences in the spectrum of disease variation (p = 1.7×10^{-4}), most notably regarding the frequencies of the *C9orf72* hexanucleotide repeat expansion (p = 3.95×10^{-4}), *SOD1* variants (p = 3.8×10^{-3}) and *TARDBP* variants (p = 0.035).

Discussion: Our results represent the most extensive account of the relative and cumulative importance of identified ALS loci to date. They reveal that ˜17% of Irish ALS patients may carry high penetrance variants within the investigated genes, with the *C9orf72* repeat expansion constituting the most common cause of disease. They also indicate that genetic susceptibility to ALS varies significantly across populations and that certain variants may not cause disease in isolation.

Conclusion: Efficient and simultaneous analysis of disease-related loci can be achieved with targeted high-throughput sequencing. Such analyses may prove highly important for the purposes of managing disease heterogeneity in clinical research settings and for identifying cases of oligogenic-based disease.

P163   A FAMILIAL ALS CASE CARRYING A NOVEL P.G147C SOD1 HETEROZYGOUS MISSENSE MUTATION

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Keywords: SOD1, mutation, G147C

Background: Approximately 20% of FALS cases carry a mutation in the *SOD1* gene. Some mutations at codon 147 have already been reported. We describe a familial case with a novel G147C missense mutation.

Case report: The index case (male), developed progressive weakness and wasting of the left hand at the age of 52, followed by motor impairment of the other hand. One year after onset the neurological exam showed mild spastic paraparesis with bilateral impairment of foot extension, weakness and wasting of both hands and brisk reflexes at lower limbs. The plantar response was absent bilaterally. The tongue was slightly hypotrophic, with evident fasciculations. The patient referred to diffuse cramps and fasciculations. Neither dysphagia and dysphonia, nor dyspnea was present. The MRI of brain and cervical spine were normal. The needle EMG showed signs of chronic and active denervation at upper and lower limbs and at the bulbar level. The FVC was 106%. Collecting the family history we found that two of the patient's brothers died from ALS respectively at the age of 46 and 50, with a spinal onset and a rapid disease course, and two siblings of 49 and 43 were alive and healthy. The patient's father died at the age of 76 from chronic kidney failure without any neurological impairment, while the mother was 83 years old and healthy. The genetic analysis on the index case revealed a point, G147C missense mutations of *SOD1*. The DNA of the other family members was not available. A diagnosis of clinically definite familial ALS with genetic confirmation was made. The patient is still alive, 14 months after the disease onset.

Discussion: To our knowledge, three other missense mutations of codon 147 of *SOD1* have already been found. Andersen and colleagues first reported a p.G147R mutation in an ALS case of Icelandic origin. No further information was available. A p.G147D mutation was described in a French patient with spinal onset at the age of 73, 9-month disease course and unknown family history. Another group reported the same mutation in some Chinese familial cases, associated with a fast disease course. Finally, a p.G147S mutation was described by an Italian group in an apparently sporadic case with bulbar onset at the age of 56 and death from respiratory failure after 8 months.

The codon 147 encodes an aminoacidic residue which is highly conserved across species. This change was predicted to alter the normal function of SOD1 protein by molecular modelling studies. Most of the cases carrying mutations of the 147 codon show a rapidly progressive course, but we need further data to establish a possible genotype–phenotype correlation. This is the first report of an ALS case carrying a p.G147C heterozygous missense mutation of *SOD1* gene.

P164 FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS WITH CYS111TYR MUTATION IN CU/ZN SUPEROXIDE DISMUTASE SHOWING WIDESPREAD LEWY BODY-LIKE HYALINE INCLUSIONS

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Background: It is known that familial amyotrophic lateral sclerosis (FALS) with abnormalities of the SOD1 gene is neuropathologically characterized by the degeneration of middle root zone of the posterior column and the presence of Lewy body-like hyaline inclusions (LBHIs) in the lower motor neurons, in addition to the involvement of the upper and lower motor neurons. We report here an autopsy case of FALS with Cys111Tyr missense mutation in exon 4 of the SOD1 gene in which no pathological data have been available.

Case report: The patient's mother died of ALS at age 66. Her first symptom was weakness of the leg muscles. Our patient (male), developed insidious muscle weakness in the right leg that progressively worsened thereafter. His gait gradually became disturbed from age 38. At age 39, he also experienced weakness in the arms and the left leg. At age 41, he presented dysarthria and dysphasia and needed tube-feeding. He became bedridden from the age of 41 years, but could communicate with facial, jaw and eye movements. At age 42, he developed difficulty in respiration. About 5 years after onset of the disease, at the age of 43 years, the patient died of respiratory failure.

Results: The main neuropathological findings of the spinal cord were a significant reduction in the number of anterior horn cells and pyramidal tract degeneration as well as degeneration of Clarke's nuclei, spinocerebellar tract and middle root zone of the posterior column. In the medulla oblongata, the hypoglossal nuclei showed mild loss and shrinkage of nerve cells. In the pons, there was neuronal shrinkage and disappearance with gliosis in the trigeminal motor and facial nuclei. As seen in hematoxylin and eosin (HE) preparations, the LBHIs were eosinophilic or slightly paler inclusions and sometimes found eosinophilic cores with peripheral halos. LBHIs were seen in the anterior horn cells, Onufrowicz nucleus, Clarke's nucleus, intermediolateral column, and posterior gray horn of the spinal cord. In addition, LBHIs were observed in the periaqueductal gray matter, nucleus raphe dorsalis, locus ceruleus, trigeminal motor nucleus, vestibular nucleus, dorsal vagal nucleus, hypoglossal nucleus, and reticular formation of the brain stem.

Discussion and conclusion: It is of particular interest that in our patient a lot of neuronal LBHIs were observed not only in the lower neurons but also in the nonmotor neurons. This would suggest the possibility that the neurons of the nonmotor system were impaired by the same morbid process that affected the motor neurons. These findings raise the question of whether the involvement of multiple systems in addition to motor neuron system can be considered to represent essential FALS lesions.

P165 CO-OCCURRENCE OF MULTIPLE SCLEROSIS (MS) AND AMYOTROPHIC LATERAL SCLEROSIS (ALS) IN A PATIENT CARRYING A P.D109Y MISSENSE MUTATION OF SOD1 GENE

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Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset degenerative disorder characterized by selective loss of both upper and lower motor neuron with a fatal course. The majority of patients have a sporadic form of the disease (sALS), while about 10% of cases have a positive family history (fALS) for ALS or frontotemporal dementia. The second commonest ALS-causing gene is *SOD1*. Multiple sclerosis (MS) is a non-Mendelian, immune-mediated, inflammatory disease of the central nervous system, characterized by demyelination and axonal degeneration, leading to progressive neurological impairment. Although some reports have described the co-occurrence of ALS and MS, this association is still unexplained. We describe a 60-year-old female with definite ALS, carrying a p.D109Y missense mutation of *SOD1,* with biochemical and radiological signs consistent with a demyelinating disease.

Case report: A 60-year-old female referred to our clinic complaining weakness at right lower limb; she first became aware of mild, progressive impairment in walking 5 years prior to admission. She reported a 45-year-old male first-degree cousin diagnosed as having MS. Neurological examination revealed pyramidal right trait alteration; she had also weakness in her right hand with hypotrophy of the first dorsal interosseus muscle. No cranial nerve involvement was observed; no cerebellar or extrapiramidal signs were present. Her deep and superficial sensation was normal. She denied urinary symptoms.

The patient underwent full laboratory tests, which were unremarkable. Needle-EMG showed active denervation in right-hand muscles; somatosensory evoked potential study was abnormal at left lower limb, absent at the right side. Motor evoked potential study showed prolonged conduction time on the right side in the cortex-L5 tract. A genetic evaluation revealed a p.D109Y missense mutation of *SOD1*; she was diagnosed as having ALS. MRI scans of the brain and cervical spine showed multiple T2-high signal periventricular lesions of the white matter and an inflammatory lesion at C1–C2 posterior cords with no abnormal enhancement. Because of this finding, she underwent a lumbar puncture, which showed oligoclonal IgG bands and elevated IgG index. Therefore, the patient satisfied McDonald's diagnostic criteria for primary progressive multiple sclerosis. She was treated with a bolus of high-dose steroids with no clinical benefit.

Discussion: According to the best of our knowledge, this is the first case of *SOD1*-ALS with co-morbid features of MS. The relationship in the pathogenetic mechanism between ALS and MS has already been described but is poorly understood. Hemminki et al (2009) found that ALS patients' offspring had an increased risk of MS and postulated that the shared familiar risk of MS with ALS suggest shared genetic basis. The possible association between these two disorders requires further investigation.

P166 A NEW MISSENSE MUTATION IN EXON 4 OF SOD1 GENE IN A PATIENT WITH SPORADIC ALS

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Keywords: SOD1, mutation, bioinformatics analyses

Background: Superoxide dismutase 1 (*SOD1*) gene is the second most common mutated gene in amyotrophic lateral sclerosis (ALS), accounting approximately for 20% of familial ALS cases and 3% of sporadic cases (1). To date, more than 160 missense mutations of *SOD1* have been reported. Here, we describe a novel missense mutation in exon 4 of the *SOD1* gene in a patient without family history of ALS.

Objective: To describe a novel *SOD1* mutation and to evaluate its impact on protein structure.

Method: After obtaining written consensus, DNA was extracted from peripheral blood and the *SOD1* gene was analysed by PCR and sequencing. The effect of the novel *SOD1* missense mutation on SOD1 protein was analyzed using PolyPhen, SIFT and PANTHER software. Modeling of the SOD1 variant was performed using the crystal structure of the normal SOD1 protein as reference.

Case report: The patient is a 78-year-old woman who at age 70 began to complain weakness of the left leg. During the subsequent years, the weakness spread to her right leg, leading the patient to be wheelchair confined. Physical and neurophysiological examination confirmed the involvement of only the lower motor neuron in lumbar-sacral district and no respiratory impairment, configuring a very slowly progressive flail-legs phenotype.

Results: Molecular analysis showed a heterozygous mutation g.1161G>A in the *SOD1* gene. The mutation of *GTG* to *ATG* at codon 118 in exon 4 determined a substitution of methionine for valine in SOD1 protein (V118M). Bioinformatics analysis demonstrated the possible damaging effect of this substitution on protein structure/function. Modeling of mutant SOD1 showed a destabilization of secondary structure in the amino-acid sequence around the residue 118, involving also His120 and His46, localized in the SOD1 catalytic site.

Discussion and conclusion: We identified a novel mutation V118M in a sporadic ALS patient. The mutation, in exon 4, involves an amino-acid residue highly conserved in different species. The V118M substitution is localized in the b-7 strand of the b-barrel. Such b-barrel mutations usually result in local perturbations able to alter the protein structure by affecting the monomer stability, or the dimer interface, or both at the same time (2). In this case, the V118M mutation can perturb the secondary structure of the protein, destabilizing also the His120 and His46, which bind copper ion in the catalytic site. The metal binding alteration is known to diminish the metal coordination and lead to altered SOD1–SOD1 interactions (3). Thus, the V118M can result both in a failure of protein folding and/or genesis of toxic intracellular aggregates.

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P167 GENETIC BACKGROUNDS OF ADULT ONSET LOWER MOTOR NEURON SYNDROME

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Keywords: lower motor neuron syndrome, adult-onset, hereditary motor neuropathy

Background: Lower motor neuron (LMN) syndrome is clinically characterized by signs of lower motor neuron dysfunction without signs of upper motor neuron (UMN). Whether LMN syndrome is a subtype of amyotrophic lateral sclerosis (ALS) or not is still a controversial matter today. Some LMN syndrome patients show relentless progression and poor prognosis with dyspnea or dysphagia similar to ALS, while the others show extremely slow clinical course different from ALS. In this retrospective study, we disclose the backgrounds of these slowly progressive LMN syndromes by means of both clinical and genetic methods.

Methods: We reviewed the medical records of 302 patients who had been diagnosed as having motor neuron disease at our institute over a period of 15 years from around 1997. Out of 302 patients, we selected patients who had been showing only LMN signs without UMN signs for more than 6years, focusing on their initial symptoms as unilateral weakness in the upper or lower extremity, bilateral weakness in the upper or lower extremities, and others. Primarily, we analyzed *SMN*, known as a causative gene for spinal muscular atrophy (SMA). This *SMN* analysis was performed for the purpose of checking the mutation of *SMN* for causing slowly progressive LMN syndrome characterized as proximal and symmetrical weakness in the lower extremities in adult cases. Subsequently, we analyzed the genes which cause hereditary motor neuropathy.

Results: One hundred and eight patients showed LMN signs without UMN signs at their first visit. However, UMN signs or acute exacerbation appeared to most of them in the follow-up, and finally 11 patients were diagnosed as having slowly progressive LMN syndromes. About their initial symptoms, 10 cases showed unilateral weakness in their upper or lower extremity, and only one case showed bilateral weakness in the lower extremities. Genetic analysis was carried out in nine cases upon their informed consent. None of them had *SMN* mutations, but five of them had the mutations in the genes including *DCTN1*, *HSPB1*, *GARS* and *TRPV4*, which are known to cause hereditary axonal motor neuropathy.

Conclusion: Our study showed that cases, clinically diagnosed as adult onset LMN syndromes, were different from SMA from both clinical and genetic aspects, and were composed of genetically heterogenic backgrounds including motor neuropathy.

P168   LOWER MOTOR NEURON DISEASE WITH PREDOMINANT RESPIRATORY FAILURE AND WITHOUT DEMENTIA CAUSED BY A NOVEL MAPT MUTATION IN AN ITALIAN KINDRED: CLINICAL, GENETIC AND PATHOLOGICAL CHARACTERIZATION

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Keywords: amyotrophyc lateral sclerosis, MAPT mutations, tauopathy

Case report: We report the genetic and clinicopathological characterization of a large Italian kindred with an adult onset progressive respiratory failure and proximal weakness of the upper limbs, and evidence of lower motor neuron degeneration.

A genome wide linkage analysis showed association to chromosome 17q21. A novel Asp348Gly mutation cosegregating with the disease was identified in the *MAPT* gene using exome sequencing.

The mutation does not act modifying tau interactions with microtubules. Human neuroblastoma cell lines overexpressing mutated Asp348Gly Tau isoforms displayed a consistent reduction in neurites length and arborisation. Neuropathology of an affected subject showed motoneuron loss and atrophy of the spinal anterior horns with accumulation of phosphorylated tau within the surviving motor neurons. The R3–R4 tau staining showed pathology similar to those observed in familial MAPT cases. Our data broaden the phenotype of tauopathies to include lower motor neuron disease and implicate the defect in the tau degradation pathway in motor neuron degeneration.

P169   PHENOTYPIC VARIABILITY ASSOCIATED WITH THE R155C VCP GENE MUTATION

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Keywords: VCP, mutation, distal lower motor neuron disease

Background: Mutations in the valosin-containing protein (*VCP*) gene, on chromosome 9p13.3-p12, were identified as the genetic cause of an unusual syndrome characterized by Inclusion body Myopathy with Paget disease and Frontotemporal Dementia (IBMPFD)(1). Mutations in the *VCP* gene have been recently reported as a cause of 1–2% of familial amyotrophic lateral sclerosis (ALS) cases (2). To date, 24 VCP mutations have been reported. Among these, 10 missense mutations have been identified in ALS cases, including five detected in both IBMPFD and ALS and five in only ALS cases. Here we report the R155C mutation in the *VCP* gene in an Italian patient with only lower motor neuron signs in a family with autosomal dominant inheritance.

Objectives: To further describe the diversity of phenotypes associated with mutations in the *VCP* gene.

Methods: All the 17 coding exons of *VCP* gene were analyzed using PCR and sequencing.

Case report: The patient is a 59-year-old woman followed for 11 years since presenting with progressive weakness of left hand beginning at age 29 and muscle cramps. Her symptom gradually progressed with prominent distal and symmetrical distribution weakness and muscle atrophy in upper and lower limbs. Currently she is unable to walk. No upper motor impairment neither bulbar nor cognitive involvement were detected. Her mother developed MND at 31 years of age with late onset bulbar signs and died due to respiratory failure at age 53. Anamnestic data revealed three likely affected relatives with evidence of an AD inheritance. No mutations were found in *SOD1*, *C9ORF72*, *TDP-43* and *FUS* genes in the patient. Molecular analysis showed a heterozygous mutation c.463C>T in the *VCP* gene (codon 155 in exon 5) determining a substitution of arginine to cysteine in the VCP protein (R155C). No DNA was available from the other affected relatives.

Discussion and conclusion: We identified the R155C mutation in a patient belonging to a family with autosomal dominant transmission MND. The mutation is known to be pathogenetic. Arginine155 in exon 5 is the most common mutational hot spot and the R155C is the most frequent mutation found in IBMPFD pedigrees. To date, this mutation has been previously found in only one familial ALS in which detailed clinical information is not available (3). Phenotypic variability associated with VCP mutations has been reported between and within families with FTD, IBM (Inclusion Body Myopathy), PDB (Paget's disease), ALS or a combination of these diseases. Our data extend the range of phenotype associated with VCP mutations to include patients with slowly progressive distal lower motor neuron disease.

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P170   SOMATIC VARIATION OF THE ATAXIN-2 CAG REPEAT IN MOTOR NEURON DISEASE

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Keywords: ataxin-2, expansion, mosaicism

Background: Expansion of the polyglutamine-encoding CAG tract in the ataxin-2 gene (*ATXN2*) to more than 33 repeats length causes spinocerebellar ataxia 2 (SCA2), and intermediate expansions of length 27–33 are overrepresented in motor neuron disease (MND) (1). Normal alleles (generally 22 or 23 repeats) and MND-associated alleles contain at least one CAA interruption (2), also coding for glutamine, whereas SCA2 tracts are pure CAG.

Objectives: Since repeat tract instability can result in somatic mosaicism, we tested the hypothesis that abnormal *ATXN2* CAG stretches may be a more common feature of MND than has been inferred from blood DNA, and that repeat tract genotype may influence selective vulnerability of characteristic brain regions to degeneration.

Methods: DNA was extracted from frozen post-mortem spinal cord, frontal cortex and cerebellum of 15 cases of classical MND, 10 cases of MND-*C9orf72* and 10 normal controls. The *ATXN2* CAG repeat tract was PCR-amplified and cDNA products from both alleles were co-sequenced in both directions. Protein expression was studied in parallel using immunohistochemistry on fixed paraffin sections.

Results: Somatic mosaicism of the *ATXN2* CAG repeat was detected in a minority of cases from all three groups. Variation generally involved repeats of 22 and 23 lengths with one or two CAA interrupts, none of which is likely to be involved in MND pathogenesis. However, in four MND cases where no mosaicism was detected, we did detect rare tract genotypes: two MND-*C9orf72* cases had a pure CAG tract of 23 lengths while one case of classical MND displayed a 34-repeat (in the SCA2 range) with one CAA interrupt. A 37-repeat with one CAA interruption was also seen in a MND-frontotemporal dementia case. Preliminary analysis suggests a tendency towards excessive clumping of ataxin-2 protein in MND spinal cord.

Discussion and conclusion: Somatic variation involving intermediate length and expanded *ATXN2* CAG repeats is unlikely to be a common feature of MND. Establishing the relevance to MND neuropathology of the 23-repeat pure CAG tracts and the full expansions requires further work.

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P171   A RARE MOTOR NEURON DELETERIOUS MISSENSE MUTATION IN THE DPYSL3 (CRMP4) GENE IS ASSOCIATED WITH ALS

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Keywords: ALS, CRMP4, risk factor

Background: The dihydropyrimidinase-like 3 (DPYSL3) or Collapsin Response Mediator Protein 4a (CRMP4a) expression is modified in neurodegeneration and is involved in several ALS-associated pathways including axonal transport, glutamate excitotoxicity and oxidative stress.

Objective: The objective of the study was to analyze CRMP4 as a risk factor for ALS.

Methods: We analyzed the *CRMP4* gene in French ALS patients (n = 468) and matched-controls (n = 394). We subsequently examined a variant in a Swedish population (184 SALS and 186 controls), and evaluated its functional effects on axonal growth and survival in motor neuron cell culture.

Results: The *rs147541241:A>G* missense mutation occurred in higher frequency among French ALS patients (Odds ratio = 2.99) but the association was not confirmed in the Swedish population. *In vitro* expression of mutated *CRMP4* in motor neurons reduced axonal growth and accelerated cell death compared to wild-type protein.

Discussion and conclusion: Thus, the association between the *rs147541241* mutation and ALS was limited to the French population, highlighting the geographic particularities of genetic influences (risks and contributors). The identified variant appears to shorten motor neuron survival through a detrimental effect on axonal growth and *CRMP4* could act as a key unifier in transduction pathways leading to neurodegeneration through effects on early axon development.

P172   MECHANISMS OF A NOVEL PHOSPHORYLATION SITE MUTATION IN PROFILIN 1

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Keywords: PFN1, microRNAs, cytoskeleton

Background: Profilin 1 is a central regulator of actin dynamics. Mutations in the gene profilin 1 (PFN1) have very recently been shown to be the cause of a subgroup of amyotrophic lateral sclerosis (ALS). Previously, we performed a large screen of US, Nordic and German familial as well as sporadic ALS and fronto-temporal dementia (FTLD) patients for PFN1 mutations in order to get further insight into the spectrum and pathogenic relevance of this gene for the complete ALS/FTLD continuum.

Methods: Four hundred and twelve familial and 260 sporadic ALS cases, as well as 16 ALS/FTLD cases from Germany, the Nordic Countries and the US were screened for PFN1 mutations. Phenotypes of patients carrying PFN1 mutations were studied, and cell biological consequences of a novel PFN1 mutation were studied.

Results: In a German ALS family we identified the novel heterozygous PFN1 mutation p.Thr109Met, which was absent in controls. This novel mutation abrogates a phosphorylation site in profilin 1. The ALS patients with mutations in PFN1 displayed spinal onset motor neuron disease without overt cognitive involvement. PFN1 mutations were absent in patients with motor neuron disease and dementia, and in patients with only FTLD. We furthermore studied the cell biological, epigenetic and biochemical consequences of our novel profilin 1 phosphorylation site mutation.

Conclusions: We provide further evidence that PFN1 mutations can cause ALS as a Mendelian dominant trait. Patients carrying PFN1 mutations reported so far represent the classical ALS end of the ALS-FTLD spectrum. The novel p.Thr109Met mutation provides additional proof-of-principle that mutant proteins involved in the regulation of cytoskeletal dynamics can cause motor neuron degeneration. Finally, we present data outlining the cell biological consequences and mechanisms of our PFN1 phosphorylation site mutation.

P173   SCREENING OF THE PFN1 GENE IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS AND IN FRONTOTEMPORAL DEMENTIA

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Keywords: PFN1, profilin1, genetics

Background: Mutations in the profilin 1 (*PFN1*) gene, encoding a protein regulating filamentous actin growth through its binding to monomeric G-actin, have been recently identified in familial amyotrophic lateral sclerosis (ALS). Functional studies performed on ALS-associated *PFN1* mutants demonstrated aggregation propensity and alterations in growth cone and cytoskeletal dynamics (1).

Objectives: To determine the effective contribution of *PFN1* mutations to sporadic ALS (SALS) in the Italian population.

Methods: We screened a large cohort of 1168 Italian SALS patients and also included 203 frontotemporal dementia (FTD) cases because of the great clinical and genetic overlap between these two neurodegenerative diseases. The three coding exons of *PFN1* were analyzed by direct sequencing.

Results: We detected the previously described p.E117G variant in one SALS patient and the novel synonymous change p.G15G in another individual, but none in a panel of 1512 control subjects. Previous screening of *PFN1* gene in ALS suggests that p.E117G likely represents a less pathogenic variant according to both frequency data in control subjects and cases, and functional experiments (1).

Discussions and conclusion: Our results suggest that *PFN1* mutations in SALS and in FTD patients are rare, at least in the Italian population.

Reference:
1. Wu *et al.* Nature 2012.

RIGHTS LINK

P174   PFN1 MUTATIONS ARE AN UNCOMMON CAUSE OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: profilin, genetics, familial amyotrophic lateral sclerosis

Background: PROFILIN 1 (*PFN1*) mutations have been reported to underlie approximately 1% of familial amyotrophic lateral sclerosis (FALS). Recently, a number of study found that these mutations are very rare in FALS, SALS (sporadic amyotrophic lateral sclerosis) and FTD.

Objective: The aim of this study was to analyze for *PFN1* mutations a large cohort of patients with FALS.

Methods and results: A complete spectrum of *PFN1* mutations was assessed using exome sequence data generated for 209 FALS patients, 69 from Italy (ITALSGEN Consortium), 92 from USA, 13 from Israel, 27 from Germany, and 8 from Canada. The patients were diagnosed in accordance with the EI Escorial revised criteria. No mutations in *PFN1* were detected.

Conclusions: Our results suggest that *PFN1* mutations in FALS are not a common cause of disease.

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P175   ANALYSIS OF HNRNP A1, A2/B1 AND A3 GENES IN ALS PATIENTS

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Keywords: mutational analysis, hnRNPA, prion-like

Background: Mutations in the prion-like domain (PrLD) of *hnRNP A1* and *A2/B1* genes were recently identified in two families with inclusion body myopathy (IBM) associated with Paget's disease of the bone (PDB), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (IBMPFD/ALS) as well as in one familial and one sporadic ALS patient (1). The biological evidence that hnRNP A1 and A2/B1 proteins are physiological TDP-43 binding partners further reinforces the importance of these genetic findings (2). The other family member hnRNP A3 was recently shown to be sequestered in p62-positive/TDP-43-negative pathological inclusions of *C9orf72*-mutated patients and, similarly to hnRNP A1 and A2/B1, hnRNPA3 protein contains a PrLD (3).

Objectives: Molecular analysis of *hnRNP A1*, *A2/B1* and *A3* genes in a cohort of 221 familial (FALS) and 622 sporadic (SALS) Italian patients.

Methods: PCR amplification and Sanger sequencing.

Results: No variants in *hnRNP A1, A2/B1* and *A3* genes were found in 113 FALS individuals with no mutations in other ALS causative genes but a novel intronic variant in *hnRNPA2/B1* (c.659-14insT) in one patient. This intronic variant was not predicted to alter *hnRNPA2B1* splicing by *in silico* analysis. The screening of the PrLD-encoding exons of the three *hnRNP* genes in an additional panel of 108 FALS with known mutations in ALS-associated genes and of 622 SALS cases failed to find any mutation.

Discussion and conclusions: Our results suggest that mutations in *hnRNP A1*, *A2/B1* and *A3* genes are rare, at least in ALS disease.

References:
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3. Mori *et al*. Acta Neuropathol 2013.

P176   HNRNPA1 AND HNRNPA2B1 MUTATIONS IN ALS AND OTHER PROTEINOPATHIES IN THE NETHERLANDS

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Keywords: DNA analysis, multisystem proteinopathy, prion-like domain

Background: Recently mutations in *hnRNPA1* and *hnRN-PA2B1* have been identified as a cause of ALS/IBMPFD (autosomal dominant multisystem proteinopathy which results in ALS, FTD, Paget's disease and/or inclusion body myositis (IBM) with TDP-43 pathology) (1). Interestingly, *hnRNPA1* and *hnRNPA2B1* code for RNA-binding proteins and both contain a prion-like domain (PrLD), which is a shared feature with other ALS genes (TDP-43, FUS, EWSR1 and TAF15).

Objective: To determine the frequency of mutations in *hnRNPA1* and *hnRNPA2B1* in a large cohort of ALS patients, FTD patients and IBM patients from The Netherlands.

Methods: DNA samples from a total of 150 familial, 1,000 sporadic ALS patients and 500 controls were obtained from an ongoing, population-based, epidemiologic study in The Netherlands. We collected samples from 30 IBM patients that were referred to the neuromuscular clinic at the UMC Utrecht. Samples were obtained from 70 familial and 100 sporadic FTD patients that were seen at the VUMC dementia clinic. Genetic sequencing of the hnRNPA1 and hnRN-PA2B1PrLD regions was determined using Sanger sequencing.

Preliminary results: After screening 1080 sporadic ALS cases and 70 familial FTD cases, we did not identify any pathogenic mutations. One potentially interesting splice variant was detected in a single case of familial FTD (splicing (uc003sxr.4:exon9:c.695-5A>G) which could perhaps affect the PrLD, follow-up of familial ALS, sporadic FTD and IBM cases is underway.

Discussion: Mutations in hnRNPA1 and hnRNPA2B1 appear to be a rare cause of ALS and FTD.

Reference:

1. Kim *et al.* Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature 2013;495:467–73.

P177   THE MOLECULAR BASIS OF ALS IN TURKEY

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Keywords: Turkey, genetics, molecular basis

Background: Recently the frequency of ALS mutations has been extensively investigated in several populations, however, a systematic analysis has not been reported in Turkey so far.

Objectives: To carry out a comprehensive genetic study in Turkish ALS patients.

Methods: A total of 362 Turkish ALS patients, 77 fALS, belonging to 54 families, and 285 sALS cases were screened for mutations in ALS genes. Patients were genotyped for SOD1 and UBQLN2 gene mutations via conventional PCR; for *C9orf72* RP-PCR and partial southern blot analyses were performed. A subset of patients was also subjected to exome sequencing. Haplotype analysis was performed on patients carrying the SOD1-D90A mutation.

Results: SOD1 (14.8%), *C9orf72* (11.0%) and UBQLN2 (3.7%) gene mutations were found to account for approximately 29.5% of fALS in Turkey. While no SOD1 mutations were shown so far in sALS patients; *C9orf72* (3.9%) and UBQLN2 (0.7%) explained 4.6% of sALS in the cohort under study. Exomic sequencing revealed FUS, OPTN, SPG11 and PLEKHG5 mutations in four families. SOD1-D90A, which is known to occur both in dominant and recessive pedigrees, was shown to behave as a recessive trait in all three Turkish families in this study. A novel haplotype other than the common Scandinavian was detected.

Discussion and conclusion: In the framework of this study, we report a systematic screening of Turkish ALS patients for disease-causing mutations. Our results indicate that SOD1, C9Orf72 and UBQLN2 mutations are important genetic causes of ALS in the Turkish population. The frequency of SOD1 is consistent with other Mediterranean countries. Although mutational frequencies may change as additional Turkish patients are screened, the comparison of the above numbers with other population-based studies reflects both the different genetic background and the more heterogeneous nature of the Turkish population.

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P178 EXTENSIVE GENETIC ANALYSIS IN A TAIWANESE COHORT WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1, TARDBP, FUS, C9ORF72, OPTN, VCP, UBQLN2,

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by relentlessly progressive loss of motor neurons. Mutations in at least 15 genes have been described to cause familial ALS (FALS), which may account for 10–20% of ALS cases.

Objectives: The aim of this study was to determine the frequency and spectrum of mutations in different genes in a Taiwanese ALS cohort of Chinese origin.

Methods: Mutational analyses of the *SOD1, TARDBP, FUS, OPTN, VCP, UBQLN2,* and *PFN1* genes were carried out by direct sequencing in 147 unrelated patients with ALS, including 29 with FALS and 118 with sporadic ALS (SALS), diagnosed with probable or definite ALS at the Neurology Service of Taipei Veterans General Hospital, Taiwan. The CAG repeat sizes in *ATXN2* and the GGGGCC hexanucleotide repeat expansion in *C9ORF72* of the patients were also investigated.

Results: Mutations have been identified in 33 of the 147 patients (22.4%), including 21 with FALS (72.4%; 21/29) and 12 with SALS (10.2%; 12/118). Among the 29 FALS patients, 8 were found to have *SOD1* mutations (p.Thr137Arg in 2 patients, and p.Leu8Val, p.Gly10Ala, p.Asp83Asn, p.Gly85Arg, p.Leu106Phe, and p.Gly138Glu in 1), 6 had *TARDBP* mutations (p.Met337Val in 4, p.Gly348Val, and p.Asn378Asp in 1), 5 had the *C9ORF72* repeat expansion, and 2 had *FUS* mutations (p.His517Asp and p.Arg521His). Among the 118 SALS cases, 4 were found to have *SOD1* mutations (p.Gly16Ser, p.Gly37Arg, p.Cys111Try, and p.Thr137Arg), 2 had *FUS* mutations (p.H517Asp and p.Arg521His), 2 had intermediate-length CAG expansions (32 and 33 CAG repeats), 2 had the *C9ORF72* repeat expansion, 1 had a *TARDBP* mutation (p.Ser375Gly), and 1 had an *OPTN* mutation (p.Leu494Trp). No patient was found to have *VCP, UBQLN2,* or *PFN1* mutation. Four of the above-mentioned mutations are novel, including p.Gly10Ala and p.Asp83Asn in *SOD1*, p.Ser375Gly in *TARDBP*, and p.Leu494Trp in *OPTN*.

Conclusion: This study clearly demonstrates the distribution and frequency of mutations in a Taiwanese ALS cohort of Chinese origin, and expands the spectrum and supports their global presence of mutations in the causative genes of ALS.

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P179 IDENTITY-BY-DESCENT IN AN IRISH ALS COHORT

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Keywords: identity-by-descent, rare variant discovery, genome-wide SNP analysis

Background: When two individuals share a common ancestor, parts of their genome show identity-by-descent (IBD), meaning that these genomic regions are identical, with the same single nucleotide polymorphisms (SNPs) and rare variants commonly inherited in both individuals. These regions are larger and more frequent in the genome, the closer the relationship is between the two individuals.

Mapping IBD regions can serve multiple purposes in the study of complex disease, two of which are highlighted here. First, if a genomic region shows a greater degree of within-patient IBD rather than showing within-control IBD, this points towards candidate disease loci. Second, the sum total of all identified IBD regions in a pair of individuals permits inference of the degree of relationship between the pair.

Results: We have mapped IBD regions in a large Irish ALS case–control genome-wide SNP dataset using the haplotype phasing programs BEAGLE and GERMLINE, and have identified a number of genomic regions that show excessive within-case IBD, hinting at possible sites harboring multiple rare ALS-causing variants which would be discoverable by targeted resequencing. We have also noted that clusters of individuals previously classified as having sporadic ALS are actually interrelated well above the population background level, at around the level of third cousins. These individuals represent affected members of the same extended pedigree and would be good candidates for disease variant discovery by exome or genome sequencing.

Discussion and conclusion: The identification of hidden relationships among apparently sporadic ALS cases challenges the distinction between familial and sporadic forms of the disease, and supports the hypothesis that genetic etiology underpins many cases of the disease.

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P180 MOLECULAR GENETIC ANALYSIS IN RUSSIAN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mutation analysis, molecular modeling, SOD1 gene

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of upper and lower motor neurons, the development of paralyses, and death from respiratory and bulbar failure.

Objective: About 10% of ALS cases are of familial. Mutations in 18 gene loci could determine ALS and more than 25 proteins are involved in ALS pathogenesis.

Methods: Two hundred and eight ALS patients (99 females and 109 males) from the Russian population were examined, including 9 patients from 8 unrelated families with a familial form of ALS. Sequence analysis was performed for the detection of *SOD1*, *TARDBP*, and *ANG* mutations. The associations with polymorphisms in *VEGF* and *APOE* were analyzed using RT-PCR and restriction analysis. Molecular modeling analysis was used to demonstrate the pathogenetic role of *SOD1* mutations.

Results: Coding *SOD1* mutations were detected in 50% of familial cases and 3% of sporadic cases of the disease. Gly16Ala, His48Arg, Leu84Val, Ans86Ser, Asp90Ala, Ser105Leu, Glu133Gly, and Leu144Phe were detected in the coding region. All of them are present in ALSoD database and led to moderate or significant changes of the SOD1 protein energy. Mutation in His49Arg increased the protein energy, and reconstruction of the respective model revealed spatial destabilization of the molecule and abnormal interaction with the metal ion inside the active center. The other seven mutations led to decrease in the protein energy and increase in the spatial stability of SOD1, which could be accompanied by increased propensity of the 'inert' mutant molecule to misfolding and cellular aggregation.

For the first time, intronic mutations c.–46C>T, c.*249T>C (*NT_011512.11) and c.169 + 50delAACAGTA (in two unrelated patients) were detected in ALS patients and were not present in 385 controls. Coding *ANG* mutations were detected in 1.5% of sporadic ALS. No coding mutations were detected in 6 exons of *TARDBP*, but c.715–126delG deletion was associated with the risk of ALS (OR = 1.53: 95% CI, 1.05–2.24; p = 0,026). The significant difference in the genotype distribution of *VEGF* was observed between ALS cases and controls ($\chi^2 = 11.1$; p = 0.004). The significant association of the -2578A/A *VEGF* genotype with ALS was observed (OR = 2.01; 95% CI, 1.12–3.65; p = 0.018). Males carrying -2578A/A had more increased risk of ALS (OR = 2.26; 95% CI 1.01–5.12; p = 0.046). Any associations with *APOE* polymorphism were not observed.

Conclusion: In the Russian population of ALS patients, the spectrum of *SOD1* and *ANG* mutations was present. High frequency of *SOD1* mutations in familial ALS was detected. The absence of coding *TARDBP* mutation was observed. Some significant associations were detected. The results of *in silico* analysis of the *SOD1* gene mutations confirm the placement of ALS within the class of the conformational diseases.

P181 CONTRIBUTION OF MAJOR AMYOTROPHIC LATERAL SCLEROSIS-RELATED GENES TO THE ETIOLOGY OF THE DISEASE IN CHINA

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Keywords: genetics; mutation; Chinese

Background: Mutations in *SOD1*, *ANG*, *TARDBP*, *FUS*, *VCP*, *C9ORF72*, and *PFN1* genes have been identified in amyotrophic lateral sclerosis patients (1,2).

Objectives: To determine the mutations in major amyotrophic lateral sclerosis (ALS)-related genes in a large cohort of Chinese familial ALS (FALS) and sporadic ALS (SALS) patients and the genotype–phenotype associations.

Methods: Screening for mutations of *SOD1*, *ANG*, *TARDBP*, *FUS*, *VCP*, *C9ORF72*, and *PFN1* genes was consecutively carried out in 20 index FALS patients, 324 SALS patients, and 245 healthy controls admitted to Peking Union Medical College Hospital. Mutation rates of *SOD1*, *ANG*, *TARDBP*, *FUS*, and *C9orf72* genes in ALS patients in different continents were calculated, and genotype–phenotype associations were analyzed by reviewing all published studies screening for mutations in these genes in ALS patients.

Results: Overall, mutations were detected in 35.0% (95%CI, 14.1%–55.9%) and 4.0% (95%CI, 1.9%–6.1%) of FALS and SALS patients, respectively. *SOD1* (25.0%) and *FUS* (10.0%) mutations account for all mutations in FALS patients, whereas *FUS* (1.9%) and *SOD1* (0.9%) were the most frequently mutated genes, followed by *TARDBP* (0.9%), and *ANG* (0.3%) in SALS patients. No mutations were detected in *C9orf72*, *VCP*, and *PFN1*. Patients with p.H46R mutation in *SOD1* gene always manifested with weakness in the legs, the lower motor neuron signs usually dominate the clinical presentation and the disease progresses very slowly, with a mean survival of more than 17 years. Patients with mutations of p.P525L, p.R495X and nonsense mutations in *FUS* gene are associated with an early onset, a rapid disease progression, and short lifespan.

Discussion: The profile of major ALS-related genes mutations in Chinese ALS patients appears to be different from the that reported in Caucasian, in which *C9orf72* and *SOD1* are the most common mutated gene in both FALS and SALS patients, whereas *FUS* mutation is relatively less in Caucasian. The results suggest that there is an ethnic difference in the genetic background of ALS.

Conclusions: Mutations in major ALS-related genes are present in approximately 35.0% and 4.0% of Chinese FALS and SALS patients, respectively. *SOD1* and *FUS* are the most frequently mutated genes in FALS patients, while *FUS*, *SOD1*, and *TARDBP* are the most common mutated gene in SALS patients in China. Some characterized clinical phenotypes are associated with some specific gene mutations.

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P182   FLJ10986 IS NOT ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS IN A LARGE CHINESE COHORT

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Keywords: rs6700125, rs6690993, FLJ10986

The single nucleotide polymorphisms (SNPs) rs6700125 and rs6690993 in FLJ10986 (FGGY) were recently reported to be a susceptibility factor for sporadic amyotrophic lateral sclerosis (SALS) in Caucasian populations in genome-wide association studies (GWAS). However, no such association was observed in other independent GWAS or replication studies in a European cohort or a small sample size of Chinese patients. We performed a large case–control study in a cohort consisting of 963 SALS cases and 1039 controls to examine the association between the two reported SNPs in FGGY and ALS in Chinese patients. Our results did not indicate any association of these SNPs with SALS and suggest that these FLJ10986 SNPs do not modulate the risk of SALS in the Chinese population.

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P183   ASYMMETRICAL LATE-ONSET DISTAL HEREDITARY MOTOR NEUROPATHY IN A CHINESE FAMILY ASSOCIATED WITH AN HSPB1 MUTATION

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Keywords: dHMN, late-onset, HSPB1

Distal hereditary motor neuropathy (dHMN) is a clinically and genetically heterogeneous group of disorders characterized by progressive, pure motor axonal neuropathy. Mutations in the small heat-shock protein HSPB1 are responsible for one form of dHMN. Here, we report a Chinese family with strikingly asymmetrical late-onset dominant dHMN. Three patients spanning 2 generations (the patient, a 53-year-old man, his brother and his mother) developed asymmetrical distal limb weakness and muscular atrophy after the fourth decade. Gene testing excluded mutations in MFN2, GJB1 and HSPB8. Two affected family members had a heterozygous mis-sense mutation, cDNA 379C→T(127Arg→Trp), in the HSPB1 gene.

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P184   POLYMORPHISM RISK FACTOR STUDY IN FGGY GENE IN CHINESE SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: single nucleotide polymorphism, FGGY gene, susceptibility

Objective: Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder resulting from the degeneration of motor neurons in the brain and spinal cord. Sporadic ALS (SALS) accounts for the majority of patients. There were several single nucleotide polymorphisms (SNPs) reported be associated with ALS susceptibility in whole genome association analysis. We study polymorphism of rs6700125 in FGGY carbohydrate kinase domain containing FGGY or *FLJ10986* gene in SALS patient of Chinese Han origin.

Methods: We extracted the genomic DNA from the leukocytes of whole blood samples in 143 SALS patients and 153 normal controls. We applied two methods to analyze the samples, the first processed the asymmetric PCR in the presence of an unlabeled probe that contain the rs6700125 locus, and then genotyped the product on the LightScanner, confirming DNA sequence. The second-part genotyping was performed using a Sequenom chip-based MALDI-TOF mass spectrometry platform.

Results: Expression of rs6700125 that was strongly associated with susceptibility to SALS in different populations of European and American ancestry was not strongly associated with ALS in Chinese patients ($\chi^2 = 0.94$;OR $= 1.12$;95% CI, 0.84–1.6).

Conclusions: In Chinese people, rs6700125 in FGGY gene are not associated with ALS susceptibility.

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P185   C9ORF72 REPEAT EXPANSION IN CHINESE FAMILIAL AND SPORADIC ALS PATIENTS

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Keywords: Chinese population; C9ORF72; repeat expansions

Objective: To identify the existence and distribution of C9ORF72 hexanucleotide (GGGGCC) repeat expansions as well as of the mutation-related clinical phenotypes in the amyotrophic lateral sclerosis (ALS) population from mainland China.

Methods: DNA samples and clinical data of 920 sporadic ALS (SALS) cases and 61 familial ALS (FALS) patients were collected during 2007–2012. Fluorescent fragment-length analysis and repeat-primed PCR were applied to detect the pathological GGGGCC repeat expansions.

Results: The C9ORF72 mutation was found in 3 SALS patients (0.3%, 3/920) and 0 FALS patients (0, 0/61). All of them are male, spinal-onset at the age of 54–58years, later

bulbar-involved. The disease durations were from 15 to 40 months. The average C9ORF72 GGGGCC repeat numbers in 917 sALS patients without the C9ORF72 mutation is 3.89 ± 2.57 (range, 2–14).

Conclusions: This study clearly illustrates the existence and importance of the C9ORF72 repeat expansions in ALS population from mainland China, although the incidence was much lower than that in Western populations.

P186   IDENTIFICATION OF GGGGCC HEXAMER REPEAT IN ALS LYMPHOBLASTOID CELL LINES

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Keywords: C9ORF72, lymphoblasts, DNA

Background: Hexanucleotide GGGGCC repeat expansion within the first intron of C9ORF72 gene has been identified as a genetic cause for a significant proportion of ALS patients. A large study involving ALS cohorts from European, American, and non-white populations around the world found that the frequency of the C9ORF72 repeat expansion differed according to the geographical region. In particular, a genetic study of five European cohorts reported the C9ORF72 repeat expansion is around 26% of familial and 6% of sporadic ALS cases (1).

Method: The C9ORF72 repeat expansions are detected using repeat-primed PCR and/or Southern blotting techniques. The repeat PCR technique discriminates between the presence and absence of the expanded allele amplifying no more than 30 repeat copies; however, it does not allow the correct determination of the repeat number. On the other hand, Southern blot analysis allows the determination of the hexanucleotide repeat expansion size. The correlation between the GGGGCC repeat variation size in the C9ORF72 gene and the role of this transcript in different cell types is crucial for the understanding of the c9orf72-ALS pathology.

Results: Here, we report Southern blot analysis of B-lymphoblastoid cell lines derived from 22 ALS patients from the UK cohort. We have optimized a protocol that allows us the identification of the hexamer repeat size which ranges 3–23 kb. We have also observed that the majority of the lymphoblasts present different size of the hexamer repeat expansion indicating chromosome instability within each lymphoblastoid cell lines and underlying the genetic heteroegeneity of these cellular models.

Reference:

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P187   C9ORF72 REPEAT EXPANSIONS ARE SPECIFIC TO TDP-43 PROTEINOPATHIES

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Keywords: genetics, C9ORF72, FTLD

Background: Expansion of the hexanucleotide GGGGCC repeat (RE) in the *C9ORF72* gene has been recently reported as the main genetic cause of familial and sporadic cases of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (1,2). Although RE have been sporadically described also in other neurodegenerative diseases (3,4,5), a pathogenic role for *C9ORF72* in these conditions has yet to be determined.

Objectives: To assess the frequency of *C9ORF72* RE in patients with ALS-plus syndromes, amyloidopathies (Alzheimer's disease[AD]), tauopathies (progressive supranuclear palsy[PSP]and corticobasal degeneration[CBD]), and alpha-synucleinopathies (Parkinson's disease[PD]).

Methods: We screened a cohort comprised 9 patients with ALS-plus syndromes, 177 AD, 107 PSP, 103 CBD, and 190 PD cases. *C9ORF72* RE detection was performed using a two-step protocol including a repeat-primed PCR as already described (6).

Results: We identified RE in 2 of 9 patients with ALS-plus syndromes (22.2%). The first patient belongs to an ALS-FTLD pedigree and was diagnosed with PSP superimposed to a lower motor neuron disease. The second patient had a predominantly upper motor neuron disease, and developed 2years after the onset of motor symptoms, features suggestive of a concurrent CBD. Conversely, screening for the *C9ORF72* gene in PD, CBD, PSP, and AD cases was negative.

Discussions and conclusion: Our results indicate that *C9ORF72* is probably not involved in the pathogenesis of neurodegenerative diseases other than that of ALS and FTLD. However, the high frequency of *C9ORF72* RE in patients with ALS-plus syndromes suggests that, similarly to ALS-FTLD patients, individuals with motor neuron disease associated with extrapyramidal features should be screened for mutations in *C9ORF72* gene, independently on their family history.

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5. Beck *et al*. Am J Hum Genet 2013.
6. Ratti *et al*. Neurobiol Aging 2012.

P188 SOMATIC HETEROGENEITY IN C9ORF72 EXPANSIONS AND THE EFFECT OF REPEAT LENGTH ON C9ORF72 TRANSCRIPTION

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Keywords: C9ORF72, transcriptome, repeat expansion length

Background: Toxic gain-of-function and haploinsufficiency are both proposed mechanisms of pathogenicity in autosomal dominant *C9ORF72*-related amyotrophic lateral sclerosis (ALS). Southern hybridization allows sizing of the GGGGCC expansion.

Objectives: To develop an optimized Southern hybridization-based protocol for the detection of the *C9ORF72* expansion alongside independent quantification of size and variability in the number of repeats. Furthermore, identification of a patient with two expanded alleles of 50 ± 5 and > 2000 repeats allows investigation of the effect of repeat length on *C9ORF72* mRNA expression, independent of compensation by a normal-length allele.

Methods: Our protocol achieves our objective by blotting an internal standard band which labels a non-expanded fragment within the *C9ORF72* allele of each patient.

We have sized the *C9ORF72* expansion in 40 ALS cases using DNA extracted from lymphoblastoid cell lines, blood, and central nervous system tissue (CNS). qRT-PCR was used to measure *C9ORF72* mRNA expression in blood and lymphoblastoid cells and to determine its dependency on repeat number.

Results: We have demonstrated expansions of > 2000 repeats in the majority of ALS samples. Comparison between tissues of individual patients shows somatic heterogeneity with expansions in some CNS tissues of > 500 repeats longer than those in peripheral tissues.

Expression of C9ORF72 mRNA in blood of our patient with two expanded C9ORF72 alleles is equivalent to that of C9ORF72 mRNA in patients with a single expansion of > 2000 repeats, suggesting that the smaller allele is transcriptionally active. Supporting this conclusion, C9ORF72 mRNA levels in lymphoblastoid cells carrying an expansion of 50 ± 5 repeats were twice that in lines carrying more than 2000 repeats.

Discussion and conclusion: Somatic heterogeneity means evaluation of the pathogenicity will require sizing the expansion in the relevant tissue. However, performing both Southern hybridization and qRT-PCR in the blood cells of the same patients, we have shown that *C9ORF72* expansions of 50 ± 5 repeats are transcriptionally active. If < 50 repeats are pathogenic, as has been proposed, then this is unlikely to be via haploinsufficiency.

P189 C9ORF72 REPEAT EXPANSION SIZE CORRELATES WITH AGE OF ONSET AND CAUSES AGGRESSIVE DISEASE PROGRESSION IN FALS, BUT IS RARE IN OTHER MOTOR NEURON DISEASES

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Keywords: C9ORF72, southern blot analysis, somatic instability

Background: An intronic GGGGCC-hexanucleotide repeat expansion in C9ORF72 was found to be the most frequently mutated ALS gene. However, most of the genetic studies on C9ORF72 so far solely rely on an indirect PCR-based methodology.

Objectives: In this study, we aimed to determine the frequencies and lengths of C9ORF72 repeat expansions (C9RE) in different motor neuron disease cohorts (ALS, adult SMA, HSP, and PLS).

Methods: We determined the frequency of C9RE using PCR and Southern blot analysis (SBA) in DNA of lymphoblastoid cell lines (LCLs) and EDTA-treated blood derived from ALS, adult SMA, PLS and HSP patients. Moreover, we studied the variability of C9RE comparing multiple independently generated LCLs from the same patient. C9RE were compared to clinical phenotypes.

Results: 24.6% of 175 familial ALS patients, but only 1 out of 30 PLS cases and none out of 22 adult SMA and 61 HSP cases, respectively, were found to have a SBA-confirmed C9RE. C9RE were highly unstable and had a strong tendency to be shorter in LCLs compared to those in EDTA blood. Stability of C9RE in LCLs seemed to differ inter-individually. We detected a significant correlation between C9RE length and age of onset of disease in ALS patients.

Conclusion: We provide the first comprehensive study on SBA-based determination of C9RE in different motor neuron diseases. C9RE are not a common cause of motor neuron diseases other than ALS. Instability of C9RE lengths questions the predictive value of blood C9RE for the relevant CNS cell types. Finally, longer C9RE in patients with a higher age of onset raise the hypothesis that C9RE might expand during lifetime.

P190   CAN A SIMPLE VISUAL MRI RATING ATROPHY SCALE DISTINGUISH CARRIERS OF THE C9ORF72 GENETIC MUTATION FROM NON-CARRIERS?

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Background: Discovery of the C9ORF72 genetic mutation has confirmed the overlap between ALS and FTD with complex imaging analyses in this group revealing a distinct pattern of atrophy. Case selection for genetic testing of the C9ORF72 mutation can be difficult; family history alone is not a reliable predictor of mutation status as this mutation is also found in sporadic disease. Imaging analyses therefore show promise to differentiate between carriers and non-carriers; however, complex imaging techniques are not practical in the clinical setting.

Objective: To differentiate between mutation carriers and non-carriers of the C9ORF72 genetic mutation using a visual MRI rating scale which can be employed in a clinical setting.

Methods: MRI images of 46 participants (8 carriers, 16 non-carriers, and 22 controls) were rated in the following seven regions: anterior temporal lobe, orbitofrontal cortex, anterior cingulate, insula, basal ganglia, parietal lobe, and cerebellum. Areas of atrophy were rated on a five-point Likert scale by 2 raters blinded to the diagnosis.

Results: Mutation carriers had significantly more atrophy than controls in the parietal lobe ($p < 0.05$). In each of the other 6 regions, there was no significant difference in atrophy ratings between the groups. In contrast, non-carriers had significantly more atrophy in each region when compared to controls. On direct comparison between carriers and non-carriers, the latter group had significantly more atrophy than the former in each region except for the parietal region.

Conclusion: A simple visual rating scale is a useful clinical tool to aid in the selection of patients for C9ORF72 genetic testing. In addition, the parietal region has emerged as a potential marker of mutation status.

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P191   THE EFFECT OF HEXANUCLEOTIDE REPEAT EXPANSIONS ON C9ORF72 TRANSCRIPT LEVELS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: C9orf72, quantitative PCR, transcript-specific

Background: A massive expansion of a GGGGCC repeat in the *C9ORF72* gene is the most common genetic cause of ALS and causes FTLD and ALS-FTLD as well. *C9ORF72* has several transcript isoforms, and the function of the C9ORF72 protein is unknown. Our objective was to study *C9ORF72* expression in ALS patients with a C9ORF72 repeat expansion and in controls.

Methods: qPCR specific to *C9ORF72* variants 1, 2, and 3 (V1, V2, and V3) and all transcripts of *C9ORF72* were performed on a large cohort of blood samples and in fibroblasts of controls and repeat expansion mutation carriers.

Results: In blood and fibroblasts, the V1 transcript was the most abundantly expressed compared to V3, whereas V2 was scarcely expressed. In blood, V1 transcript levels were slightly reduced, but V3 levels were elevated in patients carrying the repeat. There was no difference in the expression of all *C9ORF72* transcripts. In fibroblasts from controls and patients from *C9ORF72* families, a similar pattern of small reduction in V1 expression and elevation in V3 expression was seen. However, in contrast to blood samples, an increase in all *C9ORF72* transcript expression was seen.

Discussion: Our results suggest that expanded GGGGCC repeats in *C9ORF72* do no prevent transcription from the affected allele and that haploinsufficiency is not the underlying disease mechanism in patients with *C9orf72* repeat expansion mutations.

P192   REDUCTION OF U11/U12 SMALL NUCLEAR RIBONUCLEOPROTEIN IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SMN, snRNA, splicing

Background: TAR-DNA binding protein 43kDa (TDP-43) has a crucial role in the pathogenesis of ALS. TDP-43 is one of the heterogeneous nuclear ribonucleoproteins and co-localizes with GEM, which is one of the nuclear bodies composed chiefly of survival motor neurons (SMNs) (1). SMN is a causative protein for spinal muscular atrophy, an infantile-onset motor neuron disease. SMN plays an important role in maturation of snRNAs which are components of spliceosome. Spliceosome is the machinery that carries out pre-mRNA splicing, and is classified into two types: the major (U1, U2, U4, U5, and U6) and the minor (U11, U12, U5, U4atac, and U6atac) spliceosomes. Depletion of SMN alters the repertoires of snRNAs by cell-type- and tissue-type-specific manner, particularly reduction of U11/U12 snRNAs in spinal cord (2). We have found that U12 snRNA decreased in affected tissues with ALS (Ishihara, reported in SFN 2012). However, it is unclear whether the reduction of the snRNAs alters the amounts and function of minor spliceosome.

Objective: To investigate whether the repertoires of U11/U12 spliceosome alter in affected neuronal tissues with amyotrophic lateral sclerosis (ALS), whether pre-mRNA splicing associates with U11/U12 spliceosome alters in ALS-affected issues.

Methods: Spliceosome is composed of snRNAs and associated proteins complex, small nuclear ribonucleotide protein (snRNP). Thus, we investigated the amount of U11/U12-type spliceosome in spinal motor neurons with ALS patients and controls (n = 4) using immunofluorescent staining technique with antibody to snRNP of 59 kDa, which is a component of minor spliceosome. Quantitative real-time RT-PCR of snRNAs and splicing efficiency of pre-mRNA were performed with RNA from cultured cells and neuronal tissues (spinal cord, motor cortex, and cerebellum as tissues with or without TDP-43 pathology) from ALS patients (n = 7–10) and control individuals (n = 9–10). Total RNA was extracted using mir-Vana miRNA isolation kit (Ambion).

Results: The fluorescence intensity of snRNP 59K was decreased to 39% in spinal motor neuron with ALS compared with that in control. The level of mRNA in which minor

spliceosome dependent intron was included significantly increased in TDP-43-depleted U-87 MG cells (218%), and the same results were obtained in the motor cortex with ALS but not in spinal cord and cerebellum.

Discussion and conclusion: The decreased amounts of snRNP 59K indicate that the reduction of U12 snRNA reduces the level of minor spliceosome. In addition, some minor spliceosome-dependent introns were retained in affected tissue with ALS. Our results suggest that decreasing the function of minor spliceosome may underline the molecular pathogenesis of ALS.

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P193   RNA PROCESSING FACTORS INTERACT WITH GGGGCC REPEAT EXPANSION RNA *IN VITRO* AND DISPLAY ALTERED LOCALISATION AND EXPRESSION C9ORF72 ALS CASES

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Keywords C9ORF72, sequestration, RNA

Background: Expanded GGGGCC repeats in intron 1 of the *C9ORF72* gene represent the most common cause of familial amyotrophic lateral sclerosis (ALS). Other neurodegenerative diseases result from expanded repeat sequences in non-coding regions raising the possibility of a common pathogenic mechanism. Toxic gain-of-function through sequestration of MBNL1 is a major factor underlying myotonic dystrophy types 1 and 2. Identification of protein binders of the GGGGCC repeat expansion therefore represents an important area of study which will help us better understand the pathogenic mechanism of *C9ORF72* ALS and potentially open new avenues for drug development in the future.

Objective: To identify GGGGCC RNA binding proteins from human neuronal cell and tissue extracts and investigate their pathological significance at both transcript and protein levels using *in vivo* models.

Methods: Biotinylated RNA molecules ($GGGGCC_5$ and $AAAAUU_5$) were incubated with SHSY-5Y total/nuclear extracts or extracts of human cerebellum. Streptavidin pulldown assays were carried out, and recovered proteins were identified using mass spectrometry (MS).

Spinal cord sections were prepared, motor neurons were isolated, and RNA was extracted and analysed using Human Exon 1.0ST GeneChip® microarrays. Data were analysed using the PUMA suite.

Formalin-fixed, paraffin-embedded slides from the cerebellum of C9ORF72 + cases (n = 3), C9ORF72- cases (n = 3) and controls (n = 3) were labelled with antibodies against

hnRNPF/H1, SRSF1 and p62 and visualised using fluorescence microscopy.

Results: Using MS from $GGGGCC_5$ pulldown experiments, 103 unique proteins were identified. Proteins binding to $GGGGCC_5$ showed significant enrichment of GO classes related to mRNA splicing/transport/stability. Comparison of MS-identified proteins and transcripts upregulated in *C9ORF72+* motor neurons revealed enrichment of similar GO classes with significant overlap of several specific factors implicated in ALS (including *hnRNPA3/PURA/FUS/TAF15*). Immunofluorescence experiments on C9ORF72+ cerebellar sections revealed cytoplasmic co-localisation between P62/SRSF1 and P62/hnRNPF/H1 in granular layer cells. SRSF1 levels were also shown by Western blot to be increased in *C9ORF72+* cases relative to control and *C9ORF72-* cases in extracts from cerebellar granular layer cells.

Discussion and conclusion: The mass spectrometry data obtained are in agreement with previous findings and significantly expands the number of identified GGGGCC binding proteins. We hypothesise that sequestration of these proteins by the expansion may trigger a cellular response in which the transcript level of these proteins or that of proteins with similar functions are upregulated in a compensatory mechanism. Cytoplasmic mislocalisation and co-localisation with P62 are consistent with *C9ORF72*-specific extra motor pathology. Under normal circumstances, SRSF1 negatively regulates its own protein expression by influencing the splicing and translation of its own transcripts. If SRSF1 function was impaired, by for example a sequestration event, we might expect to see an increase in its protein level. These data support a RNA toxicity using sequestration model of pathogenesis in *C9ORF*-related ALS.

P194   C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION PATHOLOGY IN THE OXFORD BRAIN BANK COHORT

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Keywords C9orf72, neuropathology, TDP-43

Background: Hexanucleotide repeat expansions in the gene *C9orf72* can cause amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), or both. The underlying neuropathology is a TDP-43 proteinopathy with additional characteristic TDP-43-negative ubiquitinated inclusions which are most pronounced in the cerebellar granule cell layer. Details of regional *C9orf72* expression and its correlation with neuropathology and clinical phenotype are still incompletely understood.

Objectives: To characterise the neuropathology and clinical phenotype of *C9orf72* repeat expansion positive cases from the Oxford Brain Bank.

Methods: Fifty cases of ALS, FTLD, or both were identified in the Oxford Brain Bank and screened using immunohistochemistry for p62-positive cerebellar granule cell inclusions

Positive cases underwent genetic testing for the *C9orf72* hexanucleotide repeat expansion using repeat-primed PCR (1) as well as further detailed immunohistochemistry work-up including semi-quantitative TDP-43 and p62 staining across various brain regions. RNA was extracted from cerebellum, hippocampus, frontal cortex, and spinal cord in cases with available frozen tissue, and *C9orf72* isoform-specific semi-quantitative PCR was performed and compared with age-matched controls.

Results: Eleven cases (5 ALS, 3 ALS-FTLD, and 3 FTLD) showed p62-positive and TDP-43-negative cerebellar granule cell inclusions. All tested positive for the hexanucleotide repeat expansion. Mean age of onset was 57 (range, 39–70) years and disease duration of 4.6 (range, 1–10) years. Spinal-onset ALS was more common than bulbar-onset ALS. The cognitive phenotype of dementia cases was behavioural variant (n = 4) and language variant (n = 2) frontotemporal dementia. Semi-quantitative assessment of p62 and TDP-43 pathology revealed that TDP-43 pathology but not p62-positive non-TDP pathology correlated best with the presence of motor neuron disease, while there was no difference in cases with dementia. Semi-quantitative RT-PCR detected all three major *C9orf72* transcripts in all examined regions, most abundant in the cerebellum. The *C9orf72* expansion did not lead to detectable new splice variants in the 5' end of the gene, or a significant shift in isoform ratios.

Discussion and conclusion: *C9orf72* hexanucleotide expansions are a common cause of FTLD and ALS in the Oxford brain bank; we describe clinicopathologic and genetic features of a new small cohort of FTLD and ALS caused by *C9orf72* hexanucleotide expansions. We focused on the anatomical relationship of TDP-43 pathology and non-TDP-43 pathology with clinical phenotype and differential splicing of C9orf72. The severity and distribution of the non-TDP-43 pathology as assessed by p62 staining did not predict the clinical phenotype in this small cohort. While our results argue against a significant splicing change of *C9orf72* in expansion carriers, they do not rule out reduced overall *C9orf72* transcript or protein levels.

Reference:

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P195   P62 PATHOLOGY AND THE FRONTOTEMPORAL SYNDROME OF ALS

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Background: P62-positive (TDP-43-negative) inclusions have been shown in the hippocampus, cerebellum and frontal cortex in patients with the expansion of the hexanucleotide repeat in C9orf72 and in the prefrontal cortex in a patient with another mutation. The presence of p62 (a ubiquitin-binding protein that facilitates the degradation of polyubiquinated proteins) as compared to TDP-43 inclusions in non-motor regions of sporadic ALS patients, and the

association between p62 inclusions and the presence of the frontotemporal syndrome, is currently unknown.

Objective: i) To examine the presence of p62 inclusions as a marker of non-motor neuron degeneration in sporadic ALS as compared to that in TDP-43 and ii) to examine the relation between p62-positive inclusions in non-motor regions and the frontotemporal syndrome, for example, mild cognitive/behavioural changes or frontotemporal dementia (FTD).

Methods: Post-mortem study was carried out with clinico-pathological correlations. The frontotemporal syndrome was diagnosed by an experienced clinician based on complaints of cognitive dysfunction, impairment on 'bedside' testing or proxy reports of behavioural changes. We used ALS cases from our brain bank with available paraffin blocks of the hippocampus and the spinal cord and sufficient information on cognitive and behavioural disturbances and/or the presence of dementia. Tissue blocks of the motor cortex and prefrontal cortex (PFC, Brodmann area 10), where available, were also investigated. Following tissue preparation, antibody characterisation and immunohistochemistry, a semi-quantitative analysis was performed for p62, ubiquitin, TDP-43 inclusions. The neuropathological diagnosis of frontotemporal lobar degeneration did not depend on p62 inclusions. The presence of inclusions in clinical subgroups was analysed using chi-square or Fisher's exact test. We are currently examining the C9orf72 hexanucleotide repeat expansion in the cases.

Results: Thirty-two ALS cases (34% bulbar onset; 10 familial, 22 sporadic) were examined of whom seven (22%) had a frontotemporal syndrome (mild in 1 sporadic and 2 familial patients, and consistent with FTD in 3 sporadic and 1 familial patient). The neuropathological diagnosis in all seven patients was ALS in combination with frontotemporal lobar degeneration. Compared to ALS patients without cognitive or behavioural problems, patients with the frontotemporal syndrome more often showed p62 (100 vs. 40%) and TDP-43 (100 vs. 20%) in the hippocampus; and more often p62 (100 vs. 41%), but not TDP-43, in the prefrontal cortex. The latter dissociation between TDP-43 and p62 was seen in both sporadic and familial cases, and was not observed in the spinal cord.

Discussion and conclusion: The more widespread presence of p62 inclusions compared to TDP-43 inclusions in the prefrontal cortex and the relation with the frontotemporal syndrome suggests that non-TDP-43 pathways lead to protein degradation and clinical signs in the prefrontal cortex of familial and sporadic ALS patients.

P196   STRUCTURE, BIOGENESIS, AND CLEARANCE OF ER-DERIVED INCLUSIONS GENERATED BY THE ALS-LINKED MUTANT OF VAPB

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Background: Vesicle-associated membrane protein-associated protein B(VAP-B)is a ubiquitously expressed ER-resident

tail-anchored adaptor protein implicated in inter-organellar lipid exchange, generation of membrane contact sites, and membrane traffic. Its mutant form, P56S-VAPB, has been linked to a dominantly inherited form of amyotrophic lateral sclerosis (ALS8). P56S-VAPB forms intracellular inclusions, whose role in ALS pathogenesis has not yet been elucidated. We recently showed (1) that newly synthesized mutant VAPB is normally targeted to the ER where it rapidly aggregates, generating a restructured ER domain consisting of ribbons of stacked cisternae apparently held together by the mutated cytosolic domain of VAPB. The P56S-VAPB structures are formed also when the mutant protein is expressed at physiological levels, and are continuous with the surrounding normally organized ER, which is, however, devoid of the mutant protein.

Objectives: To investigate the dynamics of inclusion generation and degradation.

Methods: We used stable HeLa-TetOff cell lines inducibly expressing a Myc-tagged version of wild-type VAPB or P56S-VAPB to establish a 'pulse-chase' protocol in which cells were first allowed to accumulate the mutant protein in the absence of Doxycyclin; to stop synthesis of VAPB, the antibiotic was reintroduced into the medium and the rate of decay of the two proteins was studied by analysing the levels of Myc-tagged proteins during this chase period.

Results: At variance with most pathological inclusion bodies (IBs), P56S-VAPB inclusions undergo turnover, and the rate of degradation of the aggregated polyubiquitinated mutant is faster than that of the wild-type protein. Degradation of the mutant protein involves extraction of P56S-VAPB from the ER membrane by the p97 ATPase, as suggested by the observation that transfection of a dominant negative p97 stabilizes mutant VAPB. Clearance of the inclusions occurs via the proteasome degradation pathway with no apparent participation of macro-autophagy (2). Involvement of the proteasome in P56S-VAPB elimination does not impair the proteasome's ability to clear a classical ERAD substrate. Autophagocytosis does not appear to be slowed in cells expressing mutant VAPB.

Discussion and conclusion: P56S-VAPB inclusions differ from other IBs, both in the mechanisms of their genesis and in those of their clearance from the cell. Our results reveal surprisingly efficient extraction from the ER and proteasomal degradation of this severely aggregated mutant protein. Furthermore, they suggest that the slow onset of P56S-linked familial ALS is not a consequence of the progressive accumulation of the mutant protein over time, and that the dominant inheritance of the mutant allele may rather be related to haploinsufficiency.

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P197   OPTINEURIN AND MYOSIN VI-ASSOCIATED CELLULAR TRAFFICKING DEFECTS IN ALS

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Keywords: optineurin, myosin VI, cellular trafficking

Background: Optineurin is a multifunctional protein that plays an important role in cellular trafficking events in conjunction with molecular motors such as Hungtinton, Rab8 and myosin VI. Optineurin in complex with myosin VI also plays a role in the trafficking of autophagosomes. Recently mutations in optineurin were identified to be a cause of familial amyotrophic lateral sclerosis (ALS), and optineurin-positive inclusions were described in sporadic ALS patient's motor neurons.

Objective: In this study, we aimed to define the role of optineurin mutations in the pathogenesis of ALS.

Methods: We analysed secretory protein trafficking in NSC-34 neuronal cell lines expressing optineurin, by using vesicular viral stomatitis protein (VSVG). Autophagy was examined using immunoblotting and immunocytochemistry for LC3. The formation of the optineurin–myosin VI motor complex was examined in sporadic ALS patient spinal cord lysates using immunoprecipitation.

Results: Immunocytochemical studies demonstrated that the ALS mutations triggered morphological changes to the expression of optineurin in neuronal cells. Wild-type (WT) optineurin and a glaucoma-causing mutant (E5OK) formed vesicular structures (defined as $< 3 \ \mu m^2$) in NSC-34 cells. In contrast, the ALS optineurin mutants (E478G, Q398X) did not form these structures, but instead resulted in diffused cytoplasmic accumulation and increased optineurin-positive inclusions (defined as $> 3 \ \mu m^2$). Furthermore, immunoprecipitation studies revealed that the ALS optineurin mutants did not bind to myosin VI, unlike WT and the Glaucoma mutant optineurin. Consistent with this observation, in neuronal cells expressing ALS mutant optineurin, protein trafficking from the Golgi to the plasma membrane was significantly inhibited in comparison with control cells expressing the WT optineurin or Glaucoma mutant. We further observed by immunoprecipitation that ALS optineurin mutations disrupt the complex formation of myosin VI with secretory proteins (VSVG). These results indicate that optineurin acts as a linker, connecting myosin VI with secretory vesicles during protein trafficking from the Golgi apparatus to plasma membrane. We also observed that ALS optineurin mutants induced ER stress and Golgi fragmentation in NSC-34 cells, consistent with the inhibition of protein trafficking via the classical secretory pathway. Furthermore, in cells expressing ALS optineurin mutants, there was an accumulation of autophagosomes, causing defective autophagy, suggesting that disruption of the optineurin-myosin VI complex impairs autophagy. Moreover, immunoprecipitation studies revealed that myosin VI did not co-precipitate with optineurin in sporadic ALS patient tissues in contrast to control patients, revealing that the optineurin-myosin VI complex is also disrupted in sporadic ALS.

Conclusion: This study demonstrates that optineurin is essential for Myosin VI motor function. ALS-causing mutations in optineurin disrupt the normal function of optineurin-myosin VI complex resulting in cellular pathogenic events. Interestingly, myosin VI motor complex defects were also present in sporadic ALS patient tissues, implicating optineurin-myosin VI trafficking defects in the pathogenesis of sporadic ALS.

P198   SOD1, TARDBP, AND FUS: A LINK BETWEEN GENE EXPRESSION LEVELS AND PROTEIN AGGREGATION IN NON-MUTATED AND MUTATED ALS PATIENTS

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Keywords: protein aggregation, mRNA, ALS patients

Background: Alterations in RNA metabolism have been shown in ALS opening new research perspectives on its pathogenesis (1). In this context, we have already demonstrated the increase in SOD1 mRNA level in SALS patients tissues compared to controls (2).

Objectives: Here in, we described SOD1, TARDBP and FUS mRNA levels and protein aggregation in peripheral blood mononuclear cells (PBMCs) of mutated, non-mutated sporadic ALS patients (SALS) and in sex- and age-matched healthy subjects.

Methods: We analyzed 70 sporadic ALS (SALS) and 70 controls for SOD1, TARDBP, FUS, using real-time PCR. We also included SALS patients mutated in SOD1 (L106F), TARDBP (A382T), and FUS (R521C).

We also performed immunofluorescence experiments to morphologically evaluate the subcellular distribution and appearance of the three proteins in lymphoblasts of mutated, non-mutated patients, and matched controls.

Results: We confirmed that SOD1 mRNA level was up-regulated in ALS patients compared to controls and, interestingly, SOD1 expression in mutated patients was higher than in non-mutated patients. We demonstrated that the higher level is determined by the mutated allele.

Immunofluorescence of lymphoblasts showed the presence of SOD1 cytoplasmatic inclusions in SOD1-mutated patient and sporadic cases. Small aggregates were also observed in patients mutated in FUS. The levels of TARDBP mRNA expression were similar between SALS and controls, but we evidenced that the TARDBP expression in the mutated ALS patient was higher than that in controls and non-mutated SALS cases. As SOD1, the higher mRNA level was regulated by the mutated allele. Large round-shaped cytoplasmatic speckles were evident in the patient with mutation in TDP-43, while smaller inclusions were present in sporadic cases.

Finally, we measured FUS mRNA level in SALS patients, in mutated cases and controls, and no difference was detectable among these groups. Immunofluorescence experiments showed a diffuse distribution of FUS protein in the nuclear compartment of lymphoblasts from all ALS cases as well as from control subjects.

Discussion and conclusion: We demonstrated that genetic mutations can impact both gene expression and protein aggregation. In fact, mutations in SOD1 and TARDBP gene directly correlate with mRNA level and distribution of the protein aggregation.

We hypothesize that FUS involvement in ALS is not related to gene expression and protein aggregation, but, as shown by the mutated patients, a distinct clinical phenotype seems to characterize cases carrying the R521C mutation (3). Only samples with different FUS mutations may clarify these data. We will amplify our study by recruiting SALS patients with different mutations in SOD1, TARDBP, and FUS genes to confirm our data.

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P199   FUNCTIONAL ANALYSES OF MOTOR NEURONS DIFFERENTIATED FROM ALS PATIENT-DERIVED-INDUCED PLURIPOTENT STEM CELLS (IPSC) WITH FUS AND SOD1 MUTATIONS

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Keywords: iPSC, pathophysiology, motor neurons

Background: For the study of functional deficiencies in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) patient-derived-induced pluripotent stem cell (iPSC) could become valuable *in vitro* disease models. As proof of principle, it has been demonstrated that motor neurons (MNs) differentiated from such ALS-iPSC indeed reproduce certain disease-specific abnormalities (1).

Objectives: The aim of the present study is to unravel pathophysiological mechanisms underlying ALS in MNs differentiated from iPSC lines carrying mutations in the SOD1 and FUS gene in comparison with each other and with iPSC derived from healthy controls.

Methods: Differentiation was induced based either on a novel approach starting from an expandable population of neural precursor cells generated from iPSC (2) or on a rather classical approach starting from iPSC (3). By this, MNs were generated after 18 or 42 days of differentiation, respectively.

Markers of differentiation were studied using qPCR and immunocytochemistry. To demonstrate that ALS-iPSC can be differentiated into functional MNs, cells were characterized by calcium-imaging and patch-clamp analysis. Calcium-imaging detected the expression of functional voltage-dependent calcium and ligand-gated channels such as glutamate-receptors. Whole-cell patch-clamp recording was used to confirm functional neuronal properties like sodium-inward currents and action potentials.

Results: We successfully differentiated ALS-iPSC and healthy control iPSC into Tuj1/SMI32/Islet1-positive MNs. These cells show basic neural properties such as steady membrane potential, $Na+ K+$ currents, spontaneous activity, and action potentials when being target of patch clamp analysis and calcium imaging. Calcium imaging further revealed responses to the application of neurotransmitters such as acetylcholine, glutamate, and GABA.

Discussion: Differentiation of patient- and control-derived iPSC into functional MNs paves the way to detailed comparison of potential mutation-specific functional properties regarding voltage and ligand-gated ion channels. These studies could shed more light on pathophysiological processes involved in disease onset and disease progression.

Conclusions: Having established a reliable strategy to differentiate functional MNs from ALS-iPSC and healthy controls, we now focus on detailed molecular physiological analysis of disease mechanisms in different ALS-related mutations.

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P200 INDUCED PLURIPOTENT STEM CELLS FROM ALS PATIENTS

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Keywords: induced pluripotent stem, gene mutation, familial ALS

Aim: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which lacks effective treatment. Induced pluripotent stem (iPS) cells are the perfect source for cell replacement therapy. We establish ALS-patient-specific iPS cell lines to study the mechanism and lay the foundation for future treatment.

Methods: We introduce four transcription factors including Oct4, Sox2, Klf4, c-Myc into dermal fibroblasts derived from three familial ALS patients carrying FUS P525L, SOD1 C11Y and SOD1 V14M mutations through retrovirus.

Results: These ALS-iPS cells display the morphology of tightly packed and flat colonies. Immunocytochemistry showed that these iPS cells express human ES cell surface antigens such as OCT4, SOX2, SSEA-4, TRA-1-81, and Nanog. Real-time PCR analysis suggested that ALS-iPS cells express human ES cell marker genes including OCT3/4; SOX2; NANOG; GDF3; FGF4; REX1; ESG1; DPPA2; DPPA4; and hTERT. Bisulfite genomic sequencing analyses showed that the CpG in the promoter regions of OCT4, NANOG, and REX1 were highly unmethylated. They have normal karyotypes of 46. Furthermore, they can form EBs by floating cultivation and differentiated into kinds of cell types. And they have the ability to form teratomas when injected into immunodeficient mice and to differentiate into three germ layers *in vivo*.

Conclusions: These ALS-iPS cells we generated have pluripotency which is similar to human ES cells. Therefore, we successfully established three familial ALS patient's specific iPS cell lines and are the first team to generate ALS-iPS cell lines of FUS gene mutation. These results lay the foundation of establishing an ALS-patient-specific iPS cell bank including all ALS patients and clinical applications.

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P201 MITOCHONDRIAL RESPIRATORY CHAIN ENZYMATIC ACTIVITIES AND UCP3 EXPRESSION IN MUSCLES OF PATIENTS WITH HEREDITARY AND SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mitochondrial dysfucntion, UCP3, muscle

Objective: We wished to verify whether mitochondrial respiratory chain dysfunction, decreased SOD1, and elevated UCP3 expression occur in the muscles of patients with sporadic and hereditary ALS.

Background: Amyotrophic lateral sclerosis is a complex neuromuscular disease characterized by progressive neurodegen-

eration of motor neurons with an unclear pathogenesis. A primary dysfunction of the mitochondrial respiratory chain (RC), more prominent at the level of CIV, has been previously reported in the muscles of ALS patients (1), but the results are controversial (2). Moreover, a dramatic increase in the mitochondrial uncoupling protein 3 (UCP3) has been reported in the muscles of ALS patients by a single study (3).

Methods: Activities of the mitochondrial RC enzymes (complex I, II, III, IV, I+III, and II+III) as well as of the matrix enzyme citrate synthase, were measured using the optimized spectrophotometric assays recently described by our group (4)in muscles from controls (n = 15), and different types of sporadic (n = 11, of which five with a predominant upper motor neuron phenotype, six with significant denervation) and hereditary ALS patients (two with a SOD1 gene mutation and three with a C9ORF72 mutation). Enzymatic activities of superoxide dismutase activity were also measured according to the previous protocols. Muscle sections of patients and controls were processed with a combined SDH/COX staining technique for the quantification of COX-negative fibers and ragged-blue fibers. The examiner evaluated over 350 fibers for each individual, blinded to the diagnosis. Protein expression of UCP3, the subunit 2 of Complex IV, and SOD1 was measured by western blotting.

Results: The activities of the mitochondrial RC enzymes did not differ between ALS patients and controls except in the coupled assay for complex II+III (p < 0.05). The frequency of COX-negative and ragged red fibers was also not different between patients and controls. Superoxide dismutase activities were specifically decreased only in patients carrying a heterozygous mutation in the SOD1 gene, but not in other ALS groups.

Expression of the muscle mitochondrial uncoupling protein 3 was variably expressed in both patients and controls, without any significant difference.

Conclusions: Our data argue against a significant mitochondrial dysfunction as a unifying pathogenetic mechanism in the muscles of sporadic and hereditary ALS patients. UCP3 is not a useful biomarker for ALS. We speculate that the isolated mild defect of the coupled assay for complex II+III could arise from a secondary reduction in coenzyme Q pools in the muscles of ALS patients, but this would require appropriate testing.

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P202   DJ-1 AND PINK1 IN SPORADIC ALS AND IN THE SOD1G93A ALS MOUSE MODEL: ROLE IN MITOCHONDRIAL DYSFUNCTION IN SKELETAL MUSCLES?

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Keywords: DJ-1, PINK1, muscle, mitochondrial dysfunction

Background: Mitochondrial dysfunction is an important mechanism in the pathogenesis of neurodegenerative diseases such as Parkinson's disease and ALS. DJ-1 and PTEN-induced putative kinase 1 (PINK1) are important proteins for the maintenance of mitochondrial function and the protection against cell death. Mutations in the genes coding for these proteins cause familial forms of PD. Recent studies have described changes in the expression of both proteins in ALS mouse models.

Objectives: While experiments in ALS mice reveal involvement of DJ-1 and PINK1 in the pathogenesis of SOD1^{G93A}-related ALS, tissue of human patients with sporadic ALS has not yet been analyzed for DJ-1 and PINK1 expression before. We therefore performed a comparative analysis in human postmortem tissue and in SOD1^{G93A} mice.

Methods: mRNA and protein expression of PINK1 and DJ-1 were studied using quantitative real-time PCR and immunohistochemistry in postmortem brain and spinal cord tissue, as well as in muscle biopsy samples of ALS patients, and in brain, spinal cord and gastrocnemius muscle of SOD1^{G93A} ALS mice at different disease stages.

Results: We found significant decreases in PINK1 and DJ-1 mRNA levels in muscle tissue of mice. PINK1 mRNA levels in human muscle tissue were also significantly decreased, and DJ-1 mRNA was reduced without reaching statistical significance. Immunohistochemistry of brain and spinal cord sections revealed neuronal up-regulation of both proteins in ALS.

Discussion and conclusion: Our results demonstrate a pathophysiological role of both proteins in mutant SOD1^{G93A} transgenic mice and in human sporadic ALS. They provide evidence for involvement of PINK1 and DJ-1 in mitochondrial dysfunction and muscle degeneration in both ALS patients and the SOD1^{G93A} mouse model. These results warrant for further development of therapeutic approaches aiming to increase PINK1 and DJ-1 expression.

P203   SOD1 MUTATION LEADS TO ALTERED METABOLIC PATHWAYS FOR ENERGY GENERATION IN ALS PATIENT FIBROBLASTS

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Keywords: SOD1, mitochondria, metabolism

Background: Metabolic dysfunction plays a key role in amyotrophic lateral sclerosis (ALS) disease progression and has been observed in several cellular and animal models of the disease (1,2). Peripheral tissues such as fibroblasts have been observed to recapitulate pathophysiological abnormalities observed in the CNS (3). The ease of obtaining and culturing of fibroblasts makes them a valuable model system to study pathophysiological processes relevant to neurodegeneration.

Objectives: Our objectives were to ascertain whether fibroblasts isolated from patients with SOD1-related familial ALS show the defects in energy metabolism reported in the CNS, and to identify the specific biochemical pathways underlying these changes.

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Methods: Using a Seahorse XF24 bioanalyser, which simultaneously measures the two major energy-producing pathways of the cell, mitochondrial respiration, and glycolysis in realtime, we assessed the effect of the I113T SOD1 mutation on fibroblast mitochondrial respiration, glycolytic flux, and ATP levels. The effect of SOD1 mutation on fibroblast mitochondrial function was assessed when switching from glucose to a more oxidative state under galactose. Using galactose as the sole sugar source forces the cell to rely on oxidative phosphorylation, enhancing any mitochondrial dysfunction observed when using glucose. The contribution of long-chain fatty acid oxidation (FAO) to mitochondrial respiration was also measured to ascertain whether this major energy pathway was dysfunctional in SOD1^{I113T} fibroblasts.

Results: SOD1 mutation caused a reduction in mitochondrial-coupled respiration and spare respiratory capacity in fibroblasts (19% and 32% respectively, $p \leq 0.05$). This caused a 35% reduction in ATP levels ($p \leq 0.05$) produced via oxidative phosphorylation. Concomitantly, mutant SOD1 fibroblasts upregulated glycolytic flux by 73% ($p \leq 0.01$) restoring cellular ATP levels. Forcing the fibroblasts to rely on oxidative phosphorylation using galactose as an energy source increased uncoupling in mutant SOD1^{I113T} fibroblasts indicating damaged mitochondria. Assessment of the contribution of FAO to total respiration suggested that FAO was reduced in SOD1 patient fibroblasts, which could be mimicked by starving the control cells of glucose.

Discussion: As in neurones, SOD1 mutation in fibroblasts leads to mitochondrial dysfunction either directly or as a product of increased glycolytic flux, the mutant SOD1 fibroblasts cannot rely on FAO to meet the required energy demands as SOD1 mutation leads to FAO metabolic dysregulation in fibroblasts.

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P204   METABOLIC SIGNATURES OF AMYOTROPHIC LATERAL SCLEROSIS: INSIGHTS INTO DISEASE PATHOGENESIS

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Background: Acidosis has recently been reported to be an important modulator of disease course in amyotrophic lateral sclerosis (ALS). Metabolic acid–base disorders are classically identified by measuring the concentrations of ions that affect H^+ concentration. Strong base and acid ion concentrations are used to determine the strong ion difference (SID) and the strong ion gap (SIG). The SIG represents the contribution of unidentified ions and is a strong predictor of mortality in individuals exposed to pathological acidosis. Pathological acidosis can be averted through several compensatory mechanisms, such as lowering acid production (e.g., inhibiting lactate synthesis from glycogen), promoting acid elimination, and re-synthesizing of acids back into substrate stores.

Objectives: The present study investigated whether SOD1^{G93A} mice display disease-related changes in pH within the CNS. In addition, we also determined whether the disease course affects the SID and the SIG, and whether ALS mice display compensatory mechanisms (ie increased glycogen storage and modifications in lysosomal alpha-glucosidase activity) to avert acidosis. Human ALS cervical spinal cord samples were also evaluated for changes in glycogen content and alpha-glucosidase activity to determine the relevance of our preclinical observations in humans.

Methods: *In vivo* pH measurements were made using the pH Optica system. A VetACE® clinical chemistry system was used to determine the concentration of ions for calculating SID and SIG. Glycogen levels were measured using an amplex red oxidase assay kit. Tissue alpha-glucosidase activities were determined using 4-MU-α-D-glucopyranoside as the artificial substrate. Human cervical spinal cord segments were obtained from nine ALS patients and seven age-matched normal individuals.

Results: ALS mice displayed progressive acidosis within the CNS. Furthermore, significant differences in SID and SIG values were also noted. Aberrant accumulation of glycogen in spinal cord and peripheral (muscle, liver, and kidney) tissues was observed in ALS mice. CNS glycogen levels significantly correlated ($r^2 = 0.7714$) with changes in pH. In addition, significant alternations in alpha-glucosidase activities were also found. Similarly, glycogen levels were significantly elevated in both grey and ventral white matters of human ALS tissue homogenates. Alternations in alpha-glucosidase activity were also found in human ALS spinal cord homogenates.

Discussion and conclusion: SOD1^{G93A} mice and ALS patients develop several metabolic abnormalities indicative of pathological acidosis. SID and SIG values observed in ALS mice strongly suggest that the development of acidosis was due in part to the presence of an unidentified anion. SIG values detected in ALS mice were highly predictive of imminent mortality. Furthermore, SOD1^{G93A} mice displayed metabolic changes indicative of a compensatory response to avert pathological acidosis. Increased glycogen and fluctuations in alpha-glucosidase activity within the spinal cord are also robust features of human ALS. Collectively, our work provides novel insight into the pathogenesis of ALS as well as potential biomarkers and targets for drug development.

P205   INCREASED EXPRESSION OF VALOCIN-CONTAINING PROTEIN IN THE SKIN OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: AN IMMUNOHISTOCHEMICAL STUDY

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Keywords: valocin-containing protein, skin, immunohistochemistry

Background: So far studies of the skin of amyotrophic lateral sclerosis (ALS) have shown unique pathological and

biochemical abnormalities in collagen, elastic fibers, and the ground substance. The lack of bedsore formation even in the terminal stages in ALS patients is considered characteristic. The valosin-containing protein (VCP) is one of the most evolutionarily conserved proteins that is ubiquitous and abundant in cells accounting for more than 1% of total cellular proteins. VCP was implicated in the pathogenesis of neurodegenerative diseases. Specifically VCP was found in the pathologic lesions in Alzheimer's disease, Parkinson's disease, ALS, and polyglutamine repeat diseases. It is unknown, however, whether VCP-positive (VCP+) structures are present in ALS patients' skin.

Objectives: We have carried out immunohistochemical study of VCP in the skin of ALS patients.

Methods: Skin biopsy specimens were taken from the left biceps from 20 sporadic ALS patients (61.0 ± 9.4 years) and 20 control subjects with other neurologic disorders (62.3 ± 9.8 years). Routine formalin-fixed paraffin-embedded 6-µm sections were immunostained according to standard techniques. A densitometric analysis was performed using an image analysis system.

Results: Numerous VCP+ cells were observed in the epidermis in ALS patients, which became more marked as ALS progressed, and a small number of cells were seen in controls. VCP immunoreactivity of VCP+ cells was markedly positive in the epidermis and moderately positive in some dermal blood vessels and glands in ALS patients. These findings became more conspicuous as ALS progressed. On the other hand, VCP+ cells of the epidermis, dermal blood vessels, and glands in control subjects showed a weak positive reaction even after repeated antigen-retrieval trials. The proportion of VCP+ cells in the epidermis in ALS patients ($64.0 \pm 8.8\%$) was significantly higher ($p < 0.001$) than in controls ($16.6 \pm 16.0\%$). There was a significant positive relationship ($r = 0.59$, $p < 0.01$) between the proportion and duration of illness in ALS patients. The optical density of VCP+ cells in the epidermis in ALS patients (17.7 ± 4.0) is markedly stronger ($p < 0.001$) than in controls (12.4 ± 4.2). A significant positive relation ($r = 0.61$, $p < 0.01$) was observed between the immunoreactivity and duration of illness in ALS patients.

Discussion and conclusion: This study provides the first demonstration of an increased expression of VCP in the skin of patients with ALS. These findings suggest that there may be a common process that leads to the accumulation of VCP in the motor neuron and the skin in patients with ALS. The data suggest that changes in VCP in ALS skin are likely to be related to the disease process and that metabolic alterations of VCP may take place in the skin of patients with ALS.

P206 CATALASE ACTIVITY IN DISTINCT PARTS OF BLOOD TISSUE OF PATIENTS WITH SPORADIC ALS

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Keywords: oxidative stress, catalase activity

Background: Oxidative stress may be a key element of sporadic amyotrophic lateral sclerosis (SALS) pathogenesis (1). Several data proved that markers of protein, lipids or nucleic acids peroxidation are increased in postmortem tissue of SALS individuals (1). Meanwhile, much less is known about enzymatic antioxidant defense mechanism in SALS patients (2–6).

Objectives: The aim of the study was to assess the activity of catalase (CAT), which is implicated in the defense against oxidative stress, in erythrocytes, plasma, or serum of SALS patients and healthy controls.

Methods: Altogether 46 SALS patients and 54 controls were enrolled in the study. CAT activity was estimated using the commercially available assay kit (Oxford Biomedical Research).

Results: CAT activity in erythrocytes of SALS patients was significantly decreased compared to that in neurologically intact controls ($p = 0.04$). CAT activity in plasma and serum was similar in both studied groups.

Discussion and conclusion: Erythrocyte CAT activity, in contrast to other parts of blood tissue, is reduced in SALS cases as compared to that in controls, which may indicate that antioxidant defense system plays a role in pathogenesis of SALS. However, it is difficult to conclude which parts of blood tissue are optimal for antioxidative enzyme activity assessments. At present, erythrocytes are suspected to be more sensitive to assess antioxidative enzyme activity because they are highly vulnerable to oxidative stress for the sake of their specialized function and structure (7).

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P207 HUMAN MESENCHYMAL STROMAL CELL INCREASES REGULATORY T LYMPHOCYTES/CD4 LYMPHOCYTES RATIO IN PBMC OF PATIENTS WITH ALS

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Keywords: mesenchymal stromal cell, regulatory T lymphocyte, Immunomodulation

Background: Although there have been several attempts to develop treatment for the amyotrophic lateral sclerosis (ALS), the progress has been unsuccessful and ALS has remained as an untreatable disease until now. However, several clinical trials are progressing to investigate the effect of human mesenchymal stromal cells (hMSCs) on ALS patients based on the reports that hMSCs can be a candidate for the treatment of intractable neurological disorder. In addition, recent studies have demonstrated that the positive effect of hMSCs on ALS may be due to their immunomodulatory properties.

Objectives: Based on this positive effect of the bone marrow-derived hMSCs (BM-hMSCs) on ALS patients, we investigated the immunomodulatory effect of BM-hMSCs on peripheral blood mononuclear cells (PBMCs) of ALS patients, focusing on the previous reports of alteration of regulatory T lymphocytes (CD4$^+$CD25^{hi}FoxP3$^+$, T$_{regs}$) slowing ALS progression.

Methods: Peripheral blood samples were donated from 20 healthy volunteers and 20 ALS patients diagnosed with definite or probable sporadic ALS. The PBMCs were separated using histoqpaue density-gradient method from whole blood samples. After separation, the PBMCs were cultured immediately with or without BM-hMSCs (5X10^4/well) in 24-well culture plates for 24 h or 72h. The ratio of BM-hMSC to PBMC was 1:10 in one well. BM-hMSCs was donated from healthy volunteer. After 24 h or 72 h of culturing, the PBMCs were isolated and the FACS analysis was performed in the isolated PBMCs.

Results: In the FACS analysis, we found that BM-hMSCs increased T$_{regs}$/total T lymphocytes ratio significantly in both healthy volunteers (HC) and ALS patients regardless of culture duration. The ratio of T$_{regs}$ to total T lymphocytes was calculated to be 0.093 ± 0.014 (mean $\pm$ SEM) in PBMCs (HC) only; 0.569 ± 0.07 in PBMCs + BM-hMSCs; 0.112 ± 0.014 in PBMCs (ALS); and 0.599 ± 0.081 in PBMCs (ALS) + BM-hMSC in 24-h culture system. The ratio of T$_{regs}$ to total T lymphocytes was calculated to be 0.340 ± 0.068 in PBMCs (HC) only; 1.155 ± 0.114 in PBMCs + BM-hMSCs; $0.291 \pm$

0.0040 in PBMCs (ALS); and 1.360 ± 0.156 in PBMCs (ALS) + BM-hMSC in 72-h culture system.

Conclusion: Our results suggest that BM-hMSCs may slow a disease progression of ALS patients via T$_{regs}$/total lymphocytes ratio elevation.

P208 IMMUNE ACTIVATION MARKER PRODUCTION BY CULTURED SPORADIC ALS (SALS) PATIENT BLOOD-DERIVED MACROPHAGES IMPLICATES PRO- AND ANTI-INFLAMMATORY MACROPHAGE ACTIVATION IN ALS PATHOGENESIS

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Keywords: immune activation, proinflammatory macrophage (M1), anti-inflammatory macrophage (M2)

Background: Macrophages (MOs) dominate sites of CNS injury promoting both injury and repair. These divergent effects may be caused by distinct MO subsets, that is, classically activated proinflammatory (M1) or alternatively activated anti-inflammatory (M2) MOs. Involvement of M1 activation and inflammation in ALS pathogenesis has been confirmed by various investigations. Our recent studies of SALS patient peripheral blood mononuclear cell gene expression showed upregulation of both M1 interferon-induced genes and M2 alternative activation genes (1). Abnormal elevation of M2 activation-associated cellular and secreted factors, MO scavenger receptor CD36 and chemokine CCL18 were also observed in the studies of ALS patient blood (2,3). These data suggest a hybrid activation state that implicates both M1 and M2 MO activation in ALS pathogenesis. The current study was performed to extend the earlier observations and evaluate production of both M1 and M2 MO activation markers in SALS patient blood.

Objectives: To assess C-reactive protein (CRP), matrix metallopeptidase-9 (MMP-9), and cytokine/chemokine secretions of cell culture from blood-derived MOs to define expression pattern of M1 and M2 activation markers in ALS.

Methods: Ten SALS patients and 10 healthy controls were recruited. Presence and levels of MO activation markers produced by blood-derived MOs were measured using ELISA after overnight culture. Results represent mean $\pm$ SED (pg/ml).

Results: After overnight culture, production of MO inflammatory markers CRP and MMP-9 were significantly elevated in sALS as compared to healthy control MO cultures (CRP: 3000 ± 611 vs. 1215 ± 363, p $= 0.0259$; MMP-9: 24822 ± 7242 vs. 4765 ± 1171, p $= 0.0231$). Significantly higher levels of M1 activation-related cytokine/chemokines, interferon-γ-inducible protein (CXCL11), IL-6, IL-8, MCP-1, and TNF-α were observed in ALS MO culture supernatants (CXCL11: 92.7 ± 41.7 vs. 6.9 ± 4.0, p $= 0.0125$; IL-6: 473 ± 182 vs.

29 ± 12, $p < 0.0001$; IL-8: 7312 ± 1193 vs. 2412 ± 695, $p = 0.0023$; MCP-1: 4933 ± 2627 vs. 757 ± 312, $p = 0.0147$; TNF-α: 62.5 ± 28.1 vs. 6.0 ± 0.8, $p = 0.0025$). Moreover, ALS MO also showed increased secretions of IL-1ra, a M2 activation marker (IL-1ra: 1280 ± 326 vs. 181 ± 61, $p = 0.0090$).

Discussion and conclusion: The current study showed a disease-associated secretion of both M1 and M2 activation markers in cell culture by blood-derived MOs from patients with sALS. These results were consistent with those of our previous studies implicating M1 and M2 MO activation in ALS pathogenesis. With M1 MO-associated inflammation potentially playing a significant role in ALS pathogenesis, it is likely that M2 MO alternative activation/anti-inflammatory responses seen in ALS may represent a negative feedback mechanism modulating inflammation-associated damage. Further investigation will be needed to characterize the role of both M1 and M2 MO activation in ALS pathogenesis.

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P209 ANDROGEN-DEPENDENT IMPAIRMENT OF MYOGENESIS IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Background: Spinal and bulbar muscular atrophy (SBMA) is an inherited neuromuscular disease caused by expansion of a polyglutamine (polyQ) tract in the androgen receptor (AR). SBMA is triggered by the interaction between polyQAR and its natural ligands, testosterone and dihydrotestosterone (DHT). SBMA is characterized by the loss of lower motor neurons and skeletal muscle fasciculations, weakness, and atrophy.

Aim and methods: To test the hypothesis that the interaction between polyQ-AR and androgens exerts cell-autonomous toxicity in skeletal muscle, we characterized the process of myogenesis and polyQ-AR expression in DHT-treated satellite cells obtained from SBMA patients and age-matched healthy control subjects.

Results: Treatment with androgens increased the size and number of myonuclei in myotubes from control subjects, but not from SBMA patients. Myotubes from SBMA patients had a reduced number of nuclei, suggesting impaired myotube fusion and altered contractile structures. The lack of anabolic effects of androgens on myotubes from SBMA patients was not due to the defects in myoblast proliferation, differentiation, or apoptosis. DHT treatment of myotubes from SBMA patients increased nuclear accumulation of polyQ-AR and decreased the expression of interleukin-4 (IL-4) when compared to that of myotubes from control subjects. Following DHT treatment, exposure of myotubes from SBMA patients with IL-4 treatment rescued myonuclear number and size to control levels. This supports the hypothesis that androgens alter the fusion process in SBMA myogenesis.

Conclusions: These results provide evidence of an androgen-dependent impairment of myogenesis in SBMA that could contribute to disease pathogenesis.

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THEME 9 *IN VIVO* EXPERIMENTAL MODELS

P210   ROLE OF ZPR1 IN MOTOR NEURON DEGENERATION AND SEVERITY OF SPINAL MUSCULAR ATROPHY

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Keywords: SMA, SMN, neurodegeneration

Background: Spinal muscular atrophy (SMA) is caused by mutations of the survival motor neurons (*SMN1*) gene. SMA is characterized by degeneration of spinal motor neurons caused by low levels of SMN protein. The copy number of the *SMN2* gene primarily influences the severity of SMA. Additional modifier genes that lie outside the SMA locus exist and one gene that could modify SMA is the zinc finger protein (*ZPR1*) gene (1,2). Currently, there is no treatment available to cure or reduce the burden of severity of SMA because of limited knowledge of modifier genes and the molecular mechanisms associated with SMA pathogenesis.

Objective: To examine the role of ZPR1 in motor neuron degeneration and severity of SMA.

Methods: *In vivo* studies using *Zpr1* knockout (3) and SMA model mice (4). *In vitro* studies using cultured primary spinal cord neurons.

Results: To test the significance of ZPR1 down-regulation in SMA, we examined the effect of reduced ZPR1 expression in mice with mild and severe SMA. We report that the reduced ZPR1 expression causes increase in the loss of motor neurons, hypermyelination in phrenic nerves, increases in respiratory distress and disease severity that reduces lifespan of SMA mice. The deficiency of SMN-containing sub-nuclear bodies correlates with the severity of SMA. ZPR1 is required for accumulation of SMN in sub-nuclear bodies. We report that ZPR1 overexpression increases the levels of SMN and promotes accumulation of SMN in sub-nuclear bodies in SMA patient fibroblasts. ZPR1 stimulates neurite growth and rescues axonal growth defects in SMN-deficient spinal cord neurons from SMA mice. These data suggest that the severity of disease correlates negatively with ZPR1 levels and ZPR1 may be a protective modifier of SMA.

Discussion and conclusion: ZPR1 deficiency causes defects in phrenic nerve that may contribute to respiratory distress and increase the severity of SMA. Because SMA patients express low levels of ZPR1, our data suggest phrenic nerve as a potential therapeutic target to reduce the burden of respiratory distress in SMA. ZPR1 overexpression elevates SMN levels, corrects the defect in nuclear accumulation of SMN in SMA patient cells and rescues the axonal growth of SMN-deficient neurons from SMA mice. These findings suggest that ZPR1 may be a protective modifier of SMA and opens new avenues for SMA therapeutics.

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P211   POSTNATAL REQUIREMENTS FOR SURVIVAL MOTOR NEURON (SMN), A PROTEIN DEFICIENT IN SPINAL MUSCULAR ATROPHY, DURING MATURATION AND REMODELING OF THE NEUROMUSCULAR SYSTEM

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Keywords: SMA, SMN, neuromuscular remodeling

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by survival motor neuron (SMN) protein deficiency due to mutations in the *SMN1* gene. Growing evidence indicates that SMA is a developmental disorder primarily affecting the neuromuscular system; however, a consequence of SMN reduction in adults with fully matured organs remains unclear.

Results: Here, we demonstrate, through the analysis of tamoxifen-inducible SMN-deficient mice, that induction of low SMN levels ubiquitously to the extent equivalent to those in severe SMA model mice does not mediate any abnormal phenotype in the mice older than P21, but prevents them from remodeling matured neuromuscular synapses after nerve injury. The mice were also unable to reconstruct fully matured myofibers following the skeletal muscle damage, while epithelialization after the skin injury was not affected. In control mice, the expression of SMN protein in the L5-S2 spinal motor neurons had significantly increased during the neuromuscular junction (NMJ) re-maturation process after crushing the sciatic nerve, but the increase was not detectable in SMN-deficient mice. Collectively, these results indicate that high SMN levels are required during neuromuscular

maturation. In fact, inducing low SMN levels in mice younger than P12 with immature neuromuscular system resulted in the muscle growth defects, NMJ degeneration, and progressive muscle weakness leading to death.

Discussion: Our findings suggest that patients with SMA receiving an SMN-increasing therapy can at least reduce their medication after reaching a certain age, although they need to re-augment their SMN levels under the conditions requiring neuromuscular remodeling, such as injury, diseases, and aging. This novel concept will contribute to designing further practical strategies for the treatment of individual patients with SMA.

P212   GENETIC BACKGROUND EFFECTS ON LIFESPAN OF DYNACTIN P150 GLUED MOUSE MODEL OF MOTOR NEURON DISEASE

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Keywords: modifiers, genetic background, mouse

Background: There are background-dependent differences in disease phenotype in transgenic mice that carry the mutated G93ASOD1 transgene. Expression of G93A hSOD1Tg in ALR, NODRag1KO, SJL or C3H backgrounds show a more severe phenotype, whereas a milder phenotype is observed in B6, B10, BALB/c and DBA inbred strains compared to the original mixed (B6xSJL) hSOD1Tg mice (1). These background differences are due to disease modifying genes. We have identified a Chromosome 17 QTL associated with increased lifespan in animals carrying alleles derived from B6 or B10 at this locus. Once identified, these modifiers would be of great interest, especially if they were shown to affect other motor neuron disease phenotypes.

The Dynactin p150[glued] mouse model is based on a slowly progressive autosomal dominant, lower motor neuron disease in humans that is linked to a mutation in the p150[Glued] subunit of the dynactin complex, which demonstrates clinical and pathologic changes of motor neuron disease (2,3).

Objectives: To examine background effects on disease phenotype in the Dynactin p150[glued] mouse model of motor neuron disease.

Methods: We developed two inbred strains (C57BL/6J and SJL/J) expressing the mutant human Dynactin p150[glued] originally bred on a mixed background of C57BL/6J and SJL/J. Onset of tremor along with survival was examined and compared between the two congenic strains. Survival was determined by the ability of the mouse to right itself within 10 seconds when placed on its back.

Results: We observed an acceleration of tremor onset (127.8 ± 37.7 days; N = 22 vs. 158.3 ± 31.1 days; N = 23) and decreased survival (286.8 ± 54.7; days; N = 19 vs. 344.6 ± 49.7; N = 11) when Dynactin p150 [glued] was bred onto the SJL/J background. There was a milder phenotype with later onset (175.3 ± 30.5 days; N = 14 vs. 158.3 ± 31.1 days; N = 23)

when Dynactin p150[glued] was bred on the C57BL/6J background compared to the original mixed B6/SJL background. Survival is not yet available for animals bred to the C57BL/6J background since these animals remain alive at 400 days old.

Discussion and conclusion: The genetic background influences phenotype in the Dynactin p150[glued] model of motor neuron degeneration similar to hSOD1-G93A transgenic mice that also have an accelerated phenotype when bred on SJL/J and a milder phenotype on the C57BL/6J. This suggests that there are genetic modifiers in both these disorders and that they may be similar. Identification of modifier genes might highlight intracellular pathways involved in motor neuron degeneration and provide new therapeutic targets.

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P213   ROLE OF ELP3 IN ALS

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Keywords: elongator, modulator, methylation

Background: Elp3 is the catalytic subunit of the elongator complex, comprised of six subunits (Elp1–Elp6). Elongator complex is present mainly in the nucleus, where it associates with the hyperphosphorylated RNA polymerase II, but its presence in the cytoplasm has been associated with other functions. We have identified a polymorphism in the ELP3 gene that is associated with ALS. Lower expression levels of Elp3 were found in the brain of individuals with the ALS at-risk genotype. Moreover, two loss-of-function mutations in the drosophila ELP3 were identified to induce profound axonal and synaptic defects, and the knockdown of Elp3 in zebrafish induced motor axonal abnormalities.

Objectives: To determine whether ELP3 is a modulator gene in ALS.

Methods: Overexpression and knock-down of Elp3 in the SOD1[G93A] mouse model of ALS and also in the SOD1A4V zebrafish model.

Results: We generated an ELP3^{-/-} mouse, embryonically lethal at E10.5. Nonetheless, ELP3[±] are viable and ELP3[±]/SOD1[G93A] mice become symptomatic earlier than SOD1[G93A] mice (100.4 ± 3.5 days vs. 114.6 ± 5.9 days). The survival of these animals is currently being monitored. We also generated an ELP3-overexpressing mouse that is now being crossed with SOD1[G93A] mice. Finally, we established a procedure for the AAV9-mediated overexpression of Elp3 in the spinal cord of

SOD1^{G93A} mice. Preliminary data show that Elp3 overexpression prolongs survival of SOD1^{G93A} mice by 13 days (145 days (AAV9:GFP) vs. 158.5 (AAV9:Elp3) days).

Discussion: Increasing the expression of ELP3 is beneficial in the SOD1G93A mouse whereas lowering ELP3 levels is deterimental, indicating that ELP3 may be a modulator of the disease. ELP3 has been shown to acetylate Histone H3, via this HAT domain. ELP3 also contains a SAM domain. The latest is involved in methylation/demethylation reactions. It is reasonable to speculate that ELP3 might regulate the transcription of certain genes by acetylation or methylation of histones H3. Further investigation is needed to clarify the role of ELP3 in ALS.

P214   LOSS OF FUNCTION C9ORF72 CAUSES MOTOR DEFICITS IN A ZEBRAFISH MODEL OF ALS

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Objective: To define the role that repeat expansions of a GGGGCC hexanucleotide sequence of the *C9orf72* gene play in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). A genetic model for ALS was developed to determine whether loss of function of the zebrafish orthologue of C9orf72 (zC9orf72) leads to abnormalities in neuronal development.

Methods: C9orf72 mRNA levels were quantified in brain and lymphoblasts derived from FTLD and ALS/FTLD patients and in zebrafish. Knockdown of the zC9orf72 in zebrafish was performed using two specific antisense morpholino oligonucleotides to block transcription. Quantifications of spontaneous swimming and tactile escape response, as well as measurements of axonal projections from the spinal cord, were performed.

Results: Significantly decreased expression of C9orf72 transcripts in brain and lymphoblasts was found in sporadic FTLD and ALS/FTLD patients with normal-size or expanded hexanucleotide repeats. The zC9orf72 is selectively expressed in the developing nervous system at developmental stages. Loss of function of the zC9orf72 transcripts causes both behavioral and cellular deficits related to locomotion without major morphological abnormalities. These deficits were rescued upon overexpression of human C9orf72 mRNA transcripts.

Discussion: Our results indicate C9orf72 haploinsufficiency could be a contributing factor in the spectrum of ALS/FTLD neurodegenerative disorders. Loss of function of the zebrafish orthologue of zC9orf72 expression in zebrafish is associated with axonal degeneration of motor neurons that can be rescued by expressing human C9orf72 mRNA, highlighting the specificity of the induced phenotype. These results reveal a pathogenic consequence of decreased C9orf72 levels, supporting a loss of function mechanism of disease.

P215   CHARACTERISATION OF A UNIQUE SOD1 MOUSE MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Background: Mutations in the superoxide dismutase 1 (*SOD1*) gene account for approximately 20% of familial amyotrophic lateral sclerosis (FALS) cases. Transgenic mouse models expressing mutant SOD1 have been crucial in furthering our understanding of amyotrophic lateral sclerosis (ALS). However, SOD1 transgenic mice overexpress the mutant protein and are therefore not direct genetic representations of human ALS. Thus, components of the resulting phenotype may arise from overexpression of the protein, rather than the effects of its mutation. Using MRC Harwell's *N*-ethyl-*N*-nitrosourea (ENU) bank of mutagenised mice, we identified a new mouse model that carries a point mutation in the endogenous mouse *Sod1* gene and expresses endogenous levels of SOD1, making it biochemically more relevant to human condition of ALS. This mutation, Sod1 D83G is identical to a FALS causative mutation found in humans (1).

Objectives: Our aim is to characterize the Sod1^{D83G} model and gain insights into the pathological mechanisms of ALS, using immunohistochemical, biochemical, and molecular analyses.

Methods: SOD1 activity and protein levels from brain homogenate have been assessed using an in-gel assay and Western blotting technique. Muscle force and motor unit number have been measured in tibialis anterior and extensor digitorum longus muscles, stimulating the sciatic nerve and the distal tendons of anesthetized mice. Motor neuron survival and neuromuscular junctions (NMJs) were evaluated using immunostaining and confocal microscopy.

Results: Since the D83 residue of SOD1 coordinates zinc, we verified that the SOD1 D83G protein is dismutase inactive and found that SOD1 D83G protein is unstable *in vivo*. In particular, SOD1 activity in brain extracts from *Sod1*$^{+/D83G}$ mice is reduced to ~50% whereas *Sod1*$^{D83G/D83G}$ are dismutase inactive. *Sod1*$^{D83G/D83G}$ also phenocopy *Sod1* null mice and develop hepatocellular carcinoma and progressive muscle denervation. Homozygote *Sod1*$^{D83G/D83G}$ mutant mice develop progressive degeneration of lower and upper motor neurons, although they do not become paralysed, unlike transgenic models of ALS. *Sod1*$^{D83G/D83G}$ mice do not develop overt inclusion pathology but show a progressive neuromuscular deterioration as demonstrated by the denervation of the NMJs.

Discussion and conclusions: The Sod1^{D83G} model demonstrates that a point mutation in the mouse *Sod1* gene is sufficient to cause degeneration of upper and lower motor neurons, and presents a unique model in which to dissect the role of mutant SOD1 gain and loss of function on motor neuron function and survival. In particular *Sod1*^{D83G} mice will be important for the examination of early stage pathological mechanism of ALS and may provide an excellent new tool for

testing disease therapeutics. Furthermore, by developing both motor neuron degeneration and hepatocellular carcinoma, this model offers the opportunity to study the link between cancer and neurodegeneration.

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P216   A NEW TRANSGENIC MOUSE MODEL BASED ON OVEREXPRESSION OF A CHMP2B MUTANT RECAPITULATES PARTS OF ALS AND FTD HALLMARKS

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Keywords: CHMP2B intron 5, mouse model, phenotyping

Background: Clinical, physiopathological and genetical cues converge to a pathological continuum between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). About 15% of FTD patients present with motoneuron disease and 30% of ALS patients experience FTD or other dementia symptoms (1). Both pathology can be either sporadic or of familial origin. Among the genes associated with these diseases, mutations in the *CHMP2B* (chromatin-modifying protein 2B) gene were found in patients with ALS, FTD and ALS-FTD. The CHMP2B[Intron5] mutant is a protein encoded by one of these mutations (2,3).

Objective: To study the clinical, pathological and genetic continuum between ALS and FTD and to obtain a model covering both diseases.

Methods: We have established a transgenic (Tg) mouse line expressing the human CHMP2B[intron5] mutant in neurons driven by the Thy1.2 mouse promoter. Mice were characterized using behavioural, biochemical, electrophysiological and immunohistochemical approaches.

Results: The CHMP2B[intron5] mice have decreased survival and show progressive neurodegenerative changes leading to motor and behavioural alterations. They show a strong expression of the mutant protein in neurons of the brain and the spinal cord, especially in the anterior and motor cortices and in motor neurons. This expression is associated with a gliosis and the presence of ubiquitin-positive inclusions.

The motor phenotype recapitulates several aspects of human ALS. Homozygous mutants exhibit severe and early locomotor impairments attested by a decrease in rotarod performance and grip strength, and gait abnormalities at 2 months of age. These impairments appear from 12 months in hemizygous mice and develop towards a final paralysis associated with muscle denervation, as assessed by electromyography. We further show behavioural defects relevant to FTD such

as stereotypes (repetitive rearing, excessive grooming) and food intake abnormalities.

Discussion: Here we report the generation of a Tg line expressing a human ALS/FTD-causing mutation that reproduces part of the ALS-FTD symptoms. Our data provide robust *in vivo* evidences of neurodegenerative mechanisms driven by the expression of the CHMP2B[intron5] mutant. Indepth analysis of the mechanisms leading to neuronal dysfunction may help to better understand the common physiopathological mechanisms leading to these two pathologies. This Tg line is a new promising model of ALS/FTD that will be useful for mechanistic and preclinical studies.

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P217   LOSS OF P62/SQSTM1 EXACERBATES MOTOR DYSFUNCTION IN A MUTANT SOD1-EXPRESSING MOUSE ALS MODEL

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Keywords: p62/SQSTM1, SOD1, autophagy

Background: Recent studies have revealed mis-sense variants and/or mutations in *SQSTM1* in familial as well as sporadic ALS. *SQSTM1* encodes p62/SQSTM1 that regulates the selective-autophagy via association with ubiquitinated misfolded proteins. It is notable that abundant p62/SQSTM1-positive inclusions in the brain are a typical pathological feature of ALS or ALS-FTD. Thus, p62/SQSTM1 might play a crucial role in maintenance and/or survival of motor neurons. However, it remains to be determined as to whether loss-of-function or gain-of-toxic-function of the p62/SQSTM1 mutants is associated with ALS. Furthermore, it has been shown that genetic inactivation of *Sqstm1* in mice results in the accumulation of hyperphosphorylated tau and neurodegeneration, and also in an accelerated presentation of aging phenotypes. However, no clear experimental evidence showing p62/SQSTM1 linking to motor neuron degeneration has been demonstrated.

Objectives: To examine whether p62/SQSTM1 plays a role in the onset and/or progression of ALS/MND that is associated with mutant SOD1 *in vivo*.

Methods: We generated SOD1[H46R] transgenic mice on a *Sqstm1*-null background by crossing *Sqstm1*-KO mice with the SOD1[H46R] mouse line expressing familial ALS-linked SOD1[H46R] under the control of inherent human *SOD1* promoter. We first generated congenic lines of both SOD1[H46R] transgenic and *Sqstm1*^{-/-} mice by each backcrossing more than 10 generations with C57BL/6N mice. Next, we produced *Sqstm1*[±]SOD1[H46R] mice by crossing male SOD1[H46R] and female *Sqstm1*[±] mice, and then generated six different genotype mice: wild-type, *Sqstm1*[±], *Sqstm1*^{-/-}, SOD1[H46R], *Sqstm1*[±]SOD1[H46R], and *Sqstm1*^{-/-}SOD1[H46R], by crossing male *Sqstm1*[±]SOD1[H46R] and female *Sqstm1*[±] mice. Body weight and survival of each animal were monitored. Motor coordination and balance were also measured using a balance-beam test to evaluate the motor dysfunction in the mice.

Results: *Sqstm1*^{-/-} mice did not show any gross abnormal phenotypes during the experimental period (~28 weeks). By contrast, SOD1[H46R] and *Sqstm1*[±]SOD1[H46R] mice both exhibited progressive motor dysfunction and paralysis with average life spans of 174.0 ± 16.4 (n = 82) and 175.3 ± 11.4 days (n = 41), respectively. Remarkably, *Sqstm1*^{-/-}SOD1[H46R] mice showed a shorter life span of 153.5 ± 8.0 days (n = 43). Survival in *Sqstm1*^{-/-}SOD1[H46R] mice was significantly shorter than that in wild-type or *Sqstm1*[±]; SOD1[H46R] mice ($p < 0.0001$, Kaplan–Meier analysis with log-rank test). Furthermore, a balance-beam test revealed that motor dysfunction in *Sqstm1*^{-/-}SOD1[H46R] mice (~17 weeks of age) occurs at an approximately 3 weeks earlier than that in SOD1[H46R] mice (~20 weeks of age).

Discussion and conclusion: These results indicate that lack of p62/SQSTM1 exacerbates motor dysfunction in SOD1[H46R] mice. Thus, loss of function of p62/SQSTM1 is, at least in part, associated with motor neuron diseases. Further characterization of these mice will clarify the implication of the p62/SQSTM1-mediated functions in SOD1 and/or SQSTM1-linked ALS.

P218   FATTY ACID PROFILE REVEALS PROFOUND ALTERATIONS OF LIPID METABOLISM IN SOD1 MICE

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Keywords: lipids, SCD-1, metabolism

Background: Lipid species are various molecules, ranging from pure energetic supplies to key structural player, and all are formed with at least one fatty acid. Reports point the lipid metabolism to the pathophysiology of ALS. Patients with dyslipidemia present extended life expectancy (1). An animal model of ALS, the SOD1 mice, reproduces the hypermetabolism trait of ALS, and high-fat diets extent their life expectancy (2). The length and the number of saturations present in the carbon chain of fatty acids relate to the management of energetic metabolism. A low activity of Stearoyl-Coa Desaturase-1 (SCD-1), an enzyme converting saturated fatty acid (SFA) to mono-unsaturated fatty acids (MUFAs) promotes peripheral axonal regeneration in mice (3).

Objectives: This study was designed to determine the tuning of energetic metabolism in SOD1 mice, at different disease stages, and the role of SCD-1 activity in ALS metabolic disorders.

Methods: SOD1 mice and mice treated with an inhibitor of SCD-1 were used in this study. After sacrifice, lipids from serum and liver were collected and extracted (Bligh and Dyer method). Lipids were transmethylated, and fatty acid methyl esters were analyzed by gas chromatography. Fatty acid profiles were compared to muscular denervation assessed by electromyography. Levels of circulating lipids were measured using enzymatic assays. Gene expressions were performed by quantitative PCR. Animal experiments followed European Union regulations and were approved by the ethical committee of the University of Strasbourg (No. AL/01/20/09/12).

Results: We found that relative SCD-1 activity is decreased in the serum and in the liver of SOD-1 mice, already at disease onset. The reduction of SCD-1 activity was accompanied by low levels of MUFA and increased susceptibility for lipid peroxidation. We noticed a reduction of triglycerides and total cholesterol in the serum and a downregulation of genes involved in the hepatic de novo lipogenesis. Pharmacological inhibition of SCD-1 reproduces the same metabolic traits found in SOD-1 mice.

Discussion and conclusion: Energetic lipids suffer changes in their composition, at the time that muscular denervation occurs in an animal model of ALS. These alterations reveal (i) a higher susceptibility for lipid peroxidation and (ii) a silencing of de novo lipogenesis in liver. Pharmacological inhibition of SCD1 reproduces these observations in a non-transgenic animal. Our study suggests a general role for SCD1 in the pathophysiology of amyotrophic lateral sclerosis.

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P219   DISEASE STAGE-SPECIFIC ALTERATIONS IN FAT METABOLISM IN THE HSOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: body mass index, fat metabolism, fatty acids

Background: While motor neurone loss and muscle atrophy are characteristic of ALS, increased whole body fat mass (adiposity) is correlated with slower disease progression. Increased metabolism in ALS results in reduced fat mass and lower body mass index (BMI), which is negatively associated with survival (1,2). In line with this, nutritional supplementation with a diet rich in protein and calories promotes weight gain and slows disease symptoms (3). The mechanisms by which increased metabolism and reduced BMI contribute to ALS pathology are unknown. Identification of aberrations to the key factors that maintain fat mass will provide greater understanding of ALS disease mechanisms.

Objective: To characterize the mechanisms underlying increased fat catabolism that occurs in ALS.

Methods: Male wild-type and hSOD1G93A transgenic mice were studied at various stages of disease progression. We assessed epididymal fat weight, levels of circulating non-esterified free fatty acids (NEFAs) and ketones, and levels of NEFAs in skeletal muscle. PCR arrays were used to determine the expression of genes encoding proteins and enzymes involved in the catabolism and mobilization of long-chain fatty acids (LCFAs), and genes that oxidize ketones. Data were analyzed using unpaired t-test.

Results: When compared to wild-type age-matched controls, hSOD1G93A mice failed to accumulate fat mass after the onset of disease symptoms (n $\geq$ 6/group). This was followed by subsequent increases in the expression of circulating ketones and skeletal muscle NEFAs (n $\geq$ 5/group). Gene expression analysis of white adipose tissue collected from hSOD1G93A mice at the onset of disease symptoms and wild-type age-matched controls indicated an increase in the catabolism and mobilization of LCFAs, and oxidation of ketones in ALS (n = 4/group).

Discussion and conclusion: We report evidence of increased LCFA catabolism and mobilization in the hSOD1G93A mouse. The inability to gain fat mass, coupled with increased breakdown and movement of fat from storage, and fat accumulation in skeletal muscle suggests that endogenous mechanisms promote the use of fat as an energy source in skeletal muscle during periods of increased energy need.

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P220   ANALYSIS OF HISTONE POST-TRANSLATIONAL MODIFICATIONS ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) ONSET AND PROGRESSION

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Keywords: epigenetic, histone post-translational modifications, DNA methylation

Background: Epigenetic changes encompass an array of molecular modifications to both DNA and chromatin, including post-translational covalent modification of histones at distinct amino acid residues on their amino (N)-terminal tails, such as acetylation, phosphorylation, and methylation. As for other neurodegenerative and neurological disorders, recent lines of evidence associate epigenetic gene regulation with ALS pathogenesis suggesting that transcriptional dysregulation may play a central role in the pathogenesis of ALS. Although the HDAC inhibitor (HDACi) phenylbutyrate is used in a phase II clinical trial for ALS, the efficacy of HDACi for ALS therapy awaits clinical confirmation. In fact although HDACi reasonably prolongs elevated transcriptional gene activity, many aspects of epigenetic gene regulation in ALS remain elusive. At present, different aspects of epigenetic gene regulation, including a global picture of histone modifications, have not been investigated. Filling this gap might have major implications for ALS disease progression and upcoming pharmacological interventions for epigenetic ALS therapy.

Objectives: The major objective of our work is to investigate whether in cellular and animal models for ALS, it is possible to highlight chromatin modifications associated with disease onset and progression and to study the epigenetic status of key genes known to be involved in ALS development.

Methods: In order to study the chromatin modifications induced by the expression of the ALS causative-genes SOD1, TDP43 and FUS, we use the following cellular and animal models to mimic the genetic alterations that cause ALS: (i) transgenic mice SOD1-G93Aand (ii) adenoviral delivery of ALS causative-gene in neuronal cells. Western blot analysis and immunohistochemistry have been performed on infected SHSY5Y and NSC34 cells, and on Tg spinal cord to draw a map as complete as possible as histone modifications associated with ALS.

Discussion: Preliminary results indicate that expression of wild-type or mutant ALS-causative genes correlates with specific alterations in histone modifications associated with transcriptional activation.

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P221 NEW INSIGHTS INTO THE MECHANISMS UNDERLYING THE PATHOGENESIS OF ALS USING FDG-PET AND 1H-MRS STUDIES *IN VIVO* AND ADENOVIRAL-MEDIATED GENE TRANSFER OF DNA/RNA-BINDING PROTEINS *IN VITRO*

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Keywords: neuropeptides, FDG-PET, MRS

Objectives: The objective of the study was to determine whether a specific novel stabilized neuropeptide, the principle active component in hyperimmune caprine sera (HICS), could elicit measurable efficacy in multiple models of ALS *in vivo* and *in vitro*, and to understand better the features of the mechanisms behind ALS.

Results: Age-matched SOD1[G93A] male and female mice(n = 20/treatment group naive/wild type [WT], naive/superoxide dismutase 1[SOD1] and hyperimmune caprine sera [HICS/SOD1]) were injected once daily (100 μg s.c.) using a double-blind experimental protocol starting from 60 days of age and continuing to the end-stage of the disease. Significant maintenance was observed in rotarod latency and grip strength, and concomitant changes were observed in several key cellular brainstem metabolites using 1H-MRS at 110 days. Delayed onset of disease and prolonged survival were also observed. In FDG-PET, SOD1 mice showed a significant reduction in brain glucose metabolism compared to normal naive/WT mice controls. HICS treatment was able to completely normalize and rescue the SOD1 glucose hypometabolism to normal levels (p < 0.048) using 100 μg s.c. b.i.d.

Discussion: In a separate study arm that constitutes work in progress, we are currently investigating the efficacy of HICS in primary cortical neurons and induced pluripotent stem cells transduced using adenoviral-mediated gene transfer (Ad) of several DNA/RNA-binding proteins that include the following: Ad-FUS, Ad-TDP-43, Ad-SOD1, and Ad-C9ORF72. Cell survival, protein mis-folding, and mechanisms implicated in neurotoxicity are being determined.

HICS may not only serve as a potential therapeutic agent but also help decipher aspects of the mechanisms that underlie the complex pathogenesis of ALS.

P222 CHARACTERIZATION OF INNATE AND ADAPTIVE IMMUNE RESPONSES IN THE HSOD1G93A-MCP1-CCR2 TRIPLE TRANSGENIC ALS MOUSE

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Keywords: neuroinflammation, cytokines, SOD1

Background: Building evidence revealed the involvement of innate and adaptive immune responses in both the spinal cord and motor cortex of ALS patients, and in mouse models of ALS at different stages of disease pathology (1). Secretion of cytokines including MCP1 (monocyte chemoattractant protein-1) has been detected in both cerebrospinal fluid and spinal cord of ALS patients and mouse models of ALS (2). Furthermore, MCP1-mediated recruitment of monocytes that express CCR2 (CC chemokine receptor 2) is supported by decreased levels of CCR2 + monocytes in the blood of ALS patients (3).

Objectives: To elucidate the cellular components and the molecular basis of innate and adaptive immune response in ALS using the hSOD1[G93A]-MCP1-CCR2 triple transgenic ALS mouse model, in which cells that express MCP1 and CCR2 are visualized and isolated based on their expression of mRFP (monomeric red fluorescent protein) and eGFP (enhanced green fluorescent protein), respectively. This approach uses MCP1 and CCR2 expression as a bait to genetically label cells of interest and to isolate them from the complex and heterogeneous structure of the brain as a pure group.

Methods: We generated hSOD1[G93A]-MCP1-CCR2 triple transgenic ALS mice, in which cells that MCP1 and CCR2 are genetically labeled in an ALS mouse model. Fluorescence-activated cell sorting (FACS) and microarray analysis are performed to evaluate the cellular identity and transcription profile of MCP1 + and CCR2 + cells located in the motor cortex and spinal cord at different disease stage.

Results: Our results demonstrate the presence of MCP1 + and CCR2 + cells in both the motor cortex and the spinal cord at different stages of disease initiation and progression. MCP1 + cells express macrophage/microglia lineage-specific markers at P30 and P60, suggesting their early contribution to pathology. Interestingly, a subset of CCR2 + cells expressed Ly6C, a maker for infiltrating monocytes. Microarray analysis of FACS-purified MCP1 + and CCR2 + cells at P30 started to reveal genes that are upregulated and downregulated with respect to increased immunologic response. Preliminary results suggest RNA post-translation modifications, B- and T-cell receptor signaling, and chemokine signaling pathways to be activated.

Discussion and conclusion: Using pure populations of MCP1 + and CCR2 + cells will reveal details of the molecular controls over initiation and progression of immunity in ALS. Cellular mechanisms involved in the immunologic response to vulnerable motor neurons will help identify novel therapeutic targets for building effective treatment strategies.

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P223   DEVELOPMENT OF A NEW CONFORMATION-SPECIFIC ANTI-SOD1 ANTIBODY (AJ10) USING A P2X4-LIKE HUMAN SOD1 PEPTIDE AS IMMUNIZING AGENT: IMPLICATIONS FOR IMMUNOTHERAPY IN ALS MICE

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Keywords: anti-SOD1 antibody, SOD1 misfolding, immunotherapy

Background: Previous reports from our laboratory showed that some anti-purinergic receptor P2X4 antibodies were able to cross-react with misfolded forms of amyotrophic lateral sclerosis (ALS)-linked mutant Cu/Zn superoxide dismutase (SOD1). Cross-reactivity could have been caused by the abnormal exposure of an epitope in the inner hydrophobic region of SOD1 that shared structural homology with the P2X4-immunizing peptide (1,2).

Objectives: To develop antibodies against human SOD1 epitope mimicked by the P2X4 immunizing peptide and evaluate its immunotherapeutic capabilities.

Methods: The human SOD1 sequence used to raise the antibody was VKVWGSIKGLTEGLHGFHVHEFGDNTAGC. The specificity of the antibody was tested using ELISA, immunoprecipitation, and immunohistochemistry in tissues from transgenic SOD1^{G93A} and SOD1^{G85R} mice. NSC34 cells overexpressing mutant SOD1 were also used. The possible therapeutic effects of this peptide used as a vaccine was investigated in low-copy SOD1^{G93A} mice (life span, 7–8 months) and evaluated using motor behavioral testing (rotarod, catwalk XT, open field, and ladder task) and histopathological analysis.

Results: AJ10 antibody recognizes mutant or misfolded forms of ALS-linked mutant but not SOD1 WT in native conformation. This was demonstrated in the cell line NSC34 expressing G93A or A4V mutant SOD1. AJ10 immunoreactivity was selectively associated with degenerating neurons, but not with glial cells from ALS mice overexpressing either mutant SOD1^{G93A} or SOD1^{G85R}. Neurons with strongly positive AJ10 immunostaining are often associated with activated microglial cells displaying neuronophagic activity. AJ10-immunopositive SOD1 aggregates were also found in human spinal cord from a patient with a SOD1-linked familial ALS. AJ10-immunoreactive mutant SOD1 conformers were localized in large intracellular protein aggregates with a filamentous amyloid-like organization by ultrastructural immunolabeling and also detected in neuronal organelles. AJ10 peptide in adjuvant was administered at distinct points.

Although AJ10 vaccination induced specific antibody response, preliminary results did not show any significant improvement of disease as a result.

Discussion and conclusion: All these data are congruent with the ability of the AJ10 antibody to recognize misfolded conformations of SOD1 shared by different ALS-linked SOD1 mutations but not with the native protein. Although positive effects of immunotherapy have been obtained with another SOD1 peptide (3), vaccination with AJ10 peptide does not improve ALS in our models. We conclude that neuronal mutant SOD1 conformers detected with AJ10 antibody may have pathogenetic relevance in the promotion of neuroinflammation and may define a new epitope in SOD1 for ALS research and therapy.

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P224   EFFECT OF THYMIC STIMULATION OF CD4+T-CELLS ON DISEASE ONSET AND PROGRESSION IN MUTANT SOD1 MICE

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Keywords: thymus, regulatory T lymphocytes, SOD1

Background: There is increasing evidence for a role of inflammation mediated by astrocytes, microglia and peripheral T cells in the progression of ALS and motor neuron death. Specifically, ablation of CD4+T cells accelerates disease course in mutant SOD1 mice, while passive transfer of CD4+T cells, particularly T regulatory cells, ameliorates progression. T cells must first migrate to the thymus where they mature into CD4+ and/or CD8+ cells. Here, we use castration to naturally activate the thymus through circulating androgen deprivation to stimulate CD4+ cells in mutant SOD1 mice.

Objectives: To investigate the effects of thymic stimulation on the disease course and neuropathology in male transgenic SOD1^{G93A} mice.

Methods: Male pre-pubescent SOD1^{G93A} mice were castrated or sham operated, and disease onset and progression were measured using body weight loss, locomotor ability and survival. Thymic stimulation was confirmed by measuring thymus weight and total, CD4+ and CD8+T-cell numbers were determined using FACS analysis of blood. Motor neuron, astrocyte and microglia were counted from spinal cords using immunohistochemistry. Androgen receptor (AR) expression

was also examined in spinal cords of mice using Western blotting and immunohistochemistry.

Results: Castration significantly reversed thymus atrophy and enhanced peripheral CD4 + T-cell numbers in SOD1[G93A] mice from pre-symptomatic disease. Despite stimulating thymic activity and CD4 + T-cell production, disease onset and progression were not significantly affected in SOD1[G93A] mice. Thymic stimulation did not prevent motor neuron loss or reactive astrocytosis in spinal cords of SOD1[G93A] mice; however, microgliosis was suppressed. We also show that AR is principally expressed on motor neurons, but not astrocytes and microglia, in spinal cord, and that AR is depleted from spinal cords of presymptomatic SOD1[G93A] mice and is exacerbated by castration.

Discussion and conclusion: These results demonstrate that augmenting thymic function and related CD4 + T-cell production confer no benefit on the disease evolution or course in mutant SOD1 mice, in contrast to passive transfer of CD4 + T-cells. We show for the first time that AR expression is diminished in spinal motor neurons mutant SOD1 mice. Reduced AR expression on spinal motor neurons which links to another motor neuron disease, spinal bulbar muscular atrophy (SBMA), may therefore be a player in ALS pathogenesis and suggests an overlap in pathogenesis of ALS and SBMA.

P225   GENDER-SPECIFIC CONTRIBUTIONS TO ALTERED MOTONEURON SIZE IN SOD1G93A MOUSE MODEL OF ALS

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Keywords: gender, anatomy, SOD1 mouse

Background: Previous studies have shown a gender bias in both ALS patients and ALS animal models, with males showing more vulnerability. Within those patients and in SOD1 mutant mice, the first motor units lost are the largest, fast twitch motor units starting with the largest fast fatigable (IIB) units and progressing to the intermediately sized fast fatigue-resistant units (IID/X), while small, slow motor units (IIA) show most resistance. In our previous electrophysiological studies on neonatal, juvenile and adult SOD1[G93A] mice, we noted a consistent alteration in input conductance in spinal motoneurons. This indicates (indirectly) that SOD1G93A motoneurons may be physically larger in size, and that size could contribute to vulnerability along with sex.

Objective: We are directly comparing spinal motoneuron morphology in male and female adult mice during the time of fast twitch motor unit loss, well in advance of symptom onset.

Methods: Two-photon imaging was performed on motoneurons in transverse slices of sacral spinal cord and neuronal reconstructions compiled with Neurolucida software. Transgenic, overexpressed SOD1[G93A] mutant, transgenic overexpressed SOD1 wild-type, and non-transgenic wild-type mice are compared at postnatal days 30 and 50. The parameters

under examination include soma surface area; soma volume; and largest soma cross sectional area; number of stem dendrites; number of dendritic nodes (branching points); dendritic surface area; and dendritic volume.

Results: We find an increase in size in SOD1[G93A] motoneurons, particularly evident in the significantly increased soma surface area, increased number of stem dendrites, and increased total dendritic length. These anatomical differences between wild-type and G93A mice are most pronounced in males. Male SOD1[G93A] motoneurons have significantly greater soma surface area and significantly more stem dendrites than SOD1WT motoneurons, while female SOD1[G93A] motoneurons have significantly greater dendritic length than their SOD1WT counterparts.

Discussion and conclusion: Motoneuron size is increased in SOD1[G93A] mice, and more prominently in males than in females. This more pronounced phenotype in males could correspond to the delayed disease onset and longer lifespan of female vs. male SOD1[G93A] mice.

P226   VISUAL ANALYSIS AND INVESTIGATION OF TIMING AND EXTENT OF SENSORY NERVOUS SYSTEM DEGENERATION IN ALS USING A NOVEL REPORTER LINE

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Keywords: sensory nervous system, dorsal root ganglia, uchl1

Background: Amyotrophic lateral sclerosis (ALS) is mainly characterized by motor neuron degeneration; however, potential involvement of the peripheral nervous system (PNS) to disease pathology has been suggested (1,2). The spatial and temporal pattern of SNS degeneration has not been studied with respect to disease progression. We recently generated a novel ALS mouse model reporter line, the hSOD1[G93A]-UeGFP mice, in which corticospinal and spinal motor neurons, together with sensory neurons, are genetically labeled with enhanced green fluorescent protein (eGFP) expression that lasts into adulthood (3). This mouse model offers, for the first time, a comparative study of peripheral neurons together with motor neurons and allows investigation of the timing and extent of potential SNS degeneration with respect to motor neuron degeneration and disease pathology.

Objectives: To investigate the timing and extent of PNS degeneration at different stages of disease, and to assess the potential importance and relevance of sensory neuropathology in ALS, by measuring both anatomical and behavioral differences.

Methods: Sensory neurodegeneration was studied by quantification of epidermal nerve density using eGFP^{+} distal peripheral nerves in the footpads of the hSOD1^{G93A}-UeGFP mice at postnatal days (P) 30, 60, 90, and 120. Based on our initial results, von Frey test was used to determine their behavioral responses at P30 and P50.

Results: Preliminary data show a progressive and significant decrease in epidermal nerve density in the hSOD1^{G93A}-UeGFP mice by P90. Von frey test also reveal a significant difference in 50% withdrawal threshold at P50 with the hSOD1^{G93A} mice displaying allodynia, further confirming early sensory defect.

Discussion and conclusion: Decrease in epidermal nerve density has been reported in skin biopsies from late-stage ALS patients (4). Our results suggest that sensory neuropathy is an early defect in ALS, and that sensory neuron degeneration parallels that of motor neurons in ALS, adding complexity to the disease.

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P227   ALSIN KO-UEGFP MICE REVEAL LACK OF CSMN LOSS, BUT AXONAL DEFECT IN THE ABSENCE OF ALSIN FUNCTION AT P500

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Keywords: alsin, upper motor neurons, CST

Background: Mutations in *ALS2* geneis found to be one of the underlying causes for juvenile primary lateral sclerosis (1), infantile onset ascending hereditary spastic paraplegia (2), and is the most common cause for autosomal recessive juvenile ALS (1). In addition, upper motor neuron signs and bulbar symptoms are often prevalent in patients with juvenile ALS. Even though Alsin KO mice show no apparent motor function defect, it is still not clear whether upper motor neurons are affected at a cellular level in this disease model.

Objectives: To visualize and assess corticospinal motor neuron (CSMN) cellular degeneration in Alsin Ko mice (3), using a novel reporter line, the Alsin KO-UeGFP, which is generated by crossbreeding of Alsin KO and UCHL1^{-eGFP} mouse models.

Methods: We generated UCHL1^{-eGFP} mice, which express eGFP selectively in CSMN in the motor cortex. Expression

of eGFP lasts up to P800 in the mouse, allowing detailed cellular analysis of CSMN in disease models with longer life-spans (4). Using quantitative measures of genetically labeled CSMN in WT-^{UeGFP} and Alsin KO-^{eGFP} mice, we investigated the timing and the extent of CSMN cellular degeneration and axonal defects at P300 and P500.

Results: Our studies suggest the absence of prominent CSMN cellular degeneration and cell loss even at 500. We determined that CSMN are preserved and lack signs of degeneration in the motor cortex of the aged Alsin KO-^{UeGFP} mice. However, there were signs of axonal degeneration at the level of pons at P500.

Discussion: It is important to visualize CSMN to assess the extent and timing of their degeneration with respect to disease pathology in mouse. Generation of Alsin KO-^{UeGFP} mouse allowed visualization of CSMN at P300 and P500, but revealed the absence of CSMN loss event at P500. However, there was axonal defect, and CST degeneration was detected at the level of pons by P500.

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P228   NEUREGULIN-1 IS ASSOCIATED WITH POSTSYNAPTIC SITES CONTACTING AFFERENT C-TYPE CHOLINERGIC TERMINALS ON LOWER MOTONEURONS: CHANGES IN MURINE MODELS OF MOTONEURON DISEASES

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Keywords: neuregulin, C-terminals, spinal muscular atrophy

Background: Pioneering ultrastructural studies have defined different types of synapses (F-, S- and C-type boutons) on lower motoneurons (MNs). C-Type boutons are large nerve terminals characteristic of somatic α-MNs and display a subsynaptic cistern adjacent to postsynaptic membrane (1). The cholinergic nature of C-terminals has been established (2), and their origin from local interneurons has been deciphered (3). In a recent study (4) concerning neuregulin-1 (NRG-1) on phrenic MNs, it has been reported that this trophic factor is expressed in cholinergic terminals synapsing these MNs and a presynaptic localization of this protein was suggested.

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Objectives: 1) To study the distribution of NRG-1 immuno-reactivity (IR) amongst different MN groups; 2) to examine the localization of NRG-1 on spinal cord MNs by immuno-electron microscopy; 3) to perform a developmental analysis of NRG-1 expression; and 4) to explore NRG-1 changes in MNs of transgenic mouse models of spinal muscular atrophy (SMA) and ALS.

Methods: Immunofluorescence was performed on spinal cord from WT, SMNΔ7 and SOD1[G93A] mice and chick embryos. Analysis was done using confocal microscopy and ultrastructural immunolabelling.

Results: In normal adult WT animals, NRG-1-IR was concentrated close to VAChT-positive cholinergic terminals around MN somata and proximal dendrites. However, a displacement of NRG-1 signal respect to presynaptic VAChT-positive spots was evidenced, suggesting a different compartmentalisation. Ultrastructural examination revealed that NRG-1 was not associated with presynaptic terminals but with postsynaptic subsynaptic cisterns corresponding to C-terminals. A similar pattern of NRG-1-IR was found in brainstem MNs with the exception of those in oculomotor nuclei, in which NRG-1 expression was much lower. In chick embryo MNs, NRG-1 is early expressed (before E6); only at advanced developmental stages, NRG-1-IR appeared concentrated in relation to synaptic sites being downregulated after axotomy. In SMNΔ7 and SOD1[G93A], mice synaptic NRG-1 spots on MNs increased early during disease progression with a subsequent fall at the end stages.

Discussion and conclusion: 1) NRG-1 is concentrated at the subsynaptic cysterns of α-MN afferent cholinergic C-terminals; 2) ALS-resistant MNs at the oculomotor nuclei show low NRG-1 expression; 3) NRG-1 is developmentally regulated and depends on the maintenance of nerve muscle interactions; 4) the transient increase in NRG-1-positive spots on MN somata during SMA or ALS progression is in concordance with the described sprouting of C-terminals in ALS (4).

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P229 NEUROINFLAMMATION IN ALS: THE COMPLEX ROLE OF P2X7 RECEPTOR

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Keywords: P2X7 receptor, neuroinflammation, NADPH oxidase

Background: Inflammation and oxidative stress play determinant roles in the pathogenesis of ALS (1). Degenerating motor neurons produce signals that activate microglia to release ROS and proinflammatory cytokines determining a vicious cycle of neurodegeneration. Extracellular ATP through P2X7 receptor constitutes a neuron-to-microglia alarm signal, and P2X7 has been shown to be implicated in ALS (2). The comprehension of the mechanisms underlying the action of P2X7 might thus be important in counteracting the progression of the disease.

Objectives: Aims of our study were to investigate: a) the effects of pharmacological modulation and genetic ablation of P2X7 activity in microglia on the main ROS-producing enzyme NADPH oxidase 2 (NOX2), a well-known player in the pathogenesis of ALS; b) the disease outcome determined by the lack of P2X7 in SOD1[G93A] mice.

Methods: For the *in vitro* study, primary microglia derived from SOD1[G93A] and P2X7^{-/-}SOD1[G93A] mice were challenged with P2X7 agonist 2'-3'-O-(benzoyl-benzoyl) ATP and specific antagonists in order to analyze NOX2 activity and related pathways. For the *in vivo* approach, hetero- and homozygous P2X7 receptor knock-out SOD1[G93A] mice were generated and analyzed for body weight, disease onset and progression, and survival.

Results: We observed that stimulation of P2X7 enhanced NOX2 activity and ROS production in SOD1[G93A] microglia. We also found that phosphorylation of ERK1/2 was augmented in ALS-microglia and there was a mutual dependency between the NOX2 and ERK1/2 pathways. All these damaging mechanisms were prevented by blocking P2X7. Despite these *in vitro* results, in P2X7^{-/-}/SOD1[G93A] mice, the clinical onset was significantly anticipated and the disease progression worsened with consistent increased astrogliosis, microgliosis, and motoneuron loss in the lumbar spinal cord of mice at end stage.

Discussion and conclusion: Altogether, these results show that although a noxious mechanism leads in isolated ALS-microglia to enhanced oxidative stress through P2X7, its constitutive deletion in SOD1[G93A] mice aggravates the pathogenesis of ALS. These findings suggest that P2X7 might have a complex role with beneficial inflammatory effects exerted in at least definite stages of the disease. This supports the emerging dual role of neuroinflammation in ALS (3) and highlights the importance of a successful time window of therapeutic intervention against it.

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P230   KNOCKING-DOWN MGLUR1 AND MGLUR5IN SOD1G93A MICE AMELIORATES SURVIVAL AND DISEASE PROGRESSION

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Keywords: G = group I metabotrophic glutamate receptors, glutamate-mediated excitotoxicity, genetic mouse model

Background: Glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS), and reduced astrocytic uptake was suggested as a cause for the increased synaptic availability of Glu (1). On the basis of our studies, we have proposed that abnormal release may represent another source for excessive extracellular Glu levels. Acting at the altered Glu release mechanisms may represent a possible strategy for new therapeutic approaches to ALS (2,3). Our previous results indicated the existence of excessive Glu release in the spinal cord of SOD1G93A mice following activation of Group I metabotropic Glureceptors (mGluR1 and mGluR5) expressed at the pre-synaptic level (4).

Objectives: To prove the positive impact of mGluR1 or mGluR5 blockade in experimental ALS.

Methods: To provide a genetic tool to evaluate the role of mGluR1 and mGluR5 in ALS, we generated mice carrying half expression of mGluR1 in the SOD1^{G93A} background, by crossing SOD1G93A mice with heterozygous Grm1$^\pm$ mice. In the same line, we also generated mice carrying half expression of mGluR5, by crossing SOD1^{G93A} mice with Grm5$^\pm$ mice. Life span, motor abilities, MN preservation, mitochondrial damage, oxidative stress markers, astrogliosis and microglia activation, receptor expression and Glu release were investigated to characterize double-mutant mice compared to the SOD1^{G93A} ALS model.

Results: SOD1^{G93A}_Grm1$^\pm$ double-mutant mice showed prolonged survival probability respect to SOD1^{G93A} mice. Accordingly, slower disease progression and improved motor performances were observed. Interestingly, knocking down mGluR1 also reduced mGluR5 expression in the spinal cord of double-mutant mice. Histological studies performed at the late symptomatic phase of the disease showed a significant reduction on MNs death in spinal cordventro-lateral horns accompanied by lower astrocyte and microglia activation and normalization of the up-regulation of metallothionein mRNA expression in SOD1^{G93A}_Grm1$^\pm$ double mutants respect to SOD1^{G93A} mice. Reduced mitochondrial damage was also observed in soma, dendrites, axons and axons terminal in double-mutant mice. Abnormal Glu release induced by the activation of Group I mGluRs was reduced in SOD1^{G93A}_Grm1$^\pm$ compared to that in SOD1^{G93A} mice. Also SOD1^{G93A}_Grm5$^\pm$ mice, lacking of mGluR5, showed remarkable prolonged survival and phenotype amelioration.

Discussion and conclusion: mGluR1 or mGluR5 down-regulation has a significant impact *in vivo* on experimental ALS. These results would provide the rationale for pharmacological approaches to ALS by selectively blocking Group I mGluRs.

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P231   CALCITONIN GENE-RELATED PEPTIDE SIGNALING INFLUENCES MOTOR SYMPTOM ONSET AND DISEASE PROGRESSION IN THE SOD1-G93A MOUSE MODEL OF ALS

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Keywords: neuropeptide, neuroinflammation, cgrp

Background: In ALS, neuroinflammation contributes to disease initiation and progression. Recently, we have shown that alterations in the subcellular distribution of the β-isoform of the neuropeptide calcitonin gene-related peptide (CGRP) in motor neurons precede astrogliosis (1), and that CGRP expression levels predict motor neuron vulnerability (2) in the SOD1^{G93A} mouse model of ALS.

Objectives: To further elucidate the possible pathogenic role of CGRP on ALS disease progression, we crossbred SOD1 mice with mice depleted of the CGRP-specific receptor component, receptor activity-modifying protein 1 (RAMP1), and monitored clinical and histological symptom development and progression under the presence and absence of functional CGRP signaling.

Methods: Survival rate, body weight, and motor functions were monitored for wild-type; RAMP1$^{-/-}$; SOD1; and SOD1:RAMP1$^{-/-}$ mice with at least 10 animals per group. In addition, motor neuron numbers, activations of astro- and microglia, and lymphocyte infiltrations were investigated in all genotypes by immunohistochemistry at different disease stages.

Results: SOD1:RAMP1$^{-/-}$ mice showed an earlier onset of hind limb motor deficits compared to SOD1:RAMP1$^{+/+}$ mice (56 ± 30 days vs. 92 ± 20 days), while overall survival was similar (median, 142 days vs. 137 days). At the histological level, advanced onset of motor neuron degeneration in the lumbar aspect of the spinal cord was present in SOD1:RAMP1$^{-/-}$ mice compared to that in SOD1:RAMP1$^{+/+}$ mice, but followed by decelerated motor neuron loss throughout disease progression. In addition, morphological activations of astrocytes and microglia, and lymphocyte infiltrations were attenuated in SOD1:RAMP1$^{-/-}$ mice, in pre-symptomatic, early

symptomatic, and end-stage as compared to SOD1:RAMP1^{+/+} mice.

Discussion and conclusion: The observed effects under suppression of CGRP signaling in SOD1 mice suggest that the secretion of CGRP by motor neurons is neuroprotective predominantly at disease-onset, possibly through stimulation of a protective neuro-inflammatory milieu. In contrast, continuous CGRP signaling on glia at later stages furthers disease progression by promoting chronic neurodestructive neuroinflammation.

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P232 ERYTHROPOIETIN MODULATES IMMUNE-INFLAMMATORY RESPONSE IN A SOD1 (G93A) MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: amyotrophic lateral sclerosis, erythropoietin, inflammation

Background: Amyotrophic lateral sclerosis (ALS) is characterized by the appearance of reactive microglia and astroglia cells, a process referred to as neuro-inflammation. The inflammatory reaction has recently received attention as an unexpected potential target for the treatment of ALS. Erythropoietin (EPO) has shown various potent neuroprotective effects, including reduction of inflammation, enhancement of survival signals, and prevention of neuronal cell death. Recently, it has been shown that recombinant human EPO (rhEPO) is able to cross the BBB, making it a good candidate to target the CNS.

Objectives: This study was undertaken to evaluate the temporal pattern of inflammation cytokine by rhEPO treatment on SOD1[G93A] mouse model of ALS.

Methods: We treated ALS model mice with vehicle only or 5 iμ of rhEPO/g of mouse, twice every other week after they were 60 days old. Mice were analysed for inflammation cytokines involvement in the ALS pathogenic process at asymptomatic stage (30and 60 days), at early symptomatic stage (90 days) and at 120 days.

Results: In the real-time quantitative PCR (qPCR) analysis, spinal cord tissue from SOD1[G93A] mice at 90 days expressed higher levels of anti-inflammatory cytokines including TGF-β, interleukin (IL)-4 and IL-10 mRNA. In addition, late-symptomatic stage at 120 days expressed higher levels of pro-inflammatory cytokines including interferon gamma (IFN)-γ, MCP-1, IL-1β, CCL5, CXCL10 and tumour necrosis factor (TNF)-α mRNA. More importantly, when injected with

rhEPO at 60 days, these anti-inflammatory cytokines (TGF-β and IL-10) were continued to the mRNA level of symptomatic stage and IL-10 was up-regulated. However, pro-inflammatory cytokines were markedly reduced from 90 days.

Discussion and conclusion: The treatment of rhEPO significantly changed temporal inflammatory cytokine in SOD1 [G93A] mouse model of ALS. These findings suggest that rhEPO was a potential therapeutic strategy through the modulation of neuro-inflammation in ALS.

P233 NEUROINFLAMMATION AND MUSCLE DENERVATION IN WOBBLER MICE

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Keywords: inflammation, denervation, microglia, astrocyte

Objective: We evaluated the temporal relationship among diaphragm denervation, phrenic nerve and corresponding cervical cord inflammation. In addition, we examined how an inflammatory immune response contributes to the balance between neuroprotection and neurotoxicity.

Background: Neuroinflammation plays a role in the pathogenesis of amyotrophic lateral sclerosis. Neuropathological hallmarks of Wobbler mice revealed cervical motor neuron degeneration and motor axonopathy. However, little is known about the role of inflammation in Wobbler mice.

Methods: Cervical cord (C4)-phrenic nerve-diaphragm unit from 20 (early symptomatic stage)-, 40 (rapid progressive stage)-, 60 (chronic stage)-day-old Wobbler mice and age-matched wild-type littermates (C57BL/6, n = 3 each) were assessed for an inflammatory immune response using quantitative RT-PCR (CD68, GFAP, BDNF, GDNF, IL-4, TNF-α, IL-1β and NOX2) and immunofluorescent histochemistry. Denervation was evaluated in diaphragm using qRT-PCR for the temporal changes in the mRNA levels of γ(fetal) and ε(adult) acethylcholine receptor (AChR) subunits. Data were analysed using two-tailed Student's t–test, and group means were plotted $\pm$ SEM; $p < 0.05$ was considered statistically significant. Differences between groups were analysed using a two-way ANOVA.

Results: Compared with WT control mice, expression of GFAP (marker of astrocytes) in cervical cord, and AchR γ subunit in diaphragm increased at 20 days. Whereas CD68 (marker of monocytes/macrophages (microglia)) was elevated in cervical cord after 40 days. The expression of CD68 was not seen in phrenic nerve. Anti-inflammatory factors, including BDNF, GDNF and IL-4, were not increased over an entire period. In contrast, the neurotoxic markers TNF-α, IL-1β and NOX2 were increased after 20 days. Immunohistochemical study showed that astrocytes secrete TNF-α in cervical cord in Wobbler mice.

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Conclusions: We suggest that activated astrocytes play an important role at the early symptomatic stage in Wobbler mice. Muscle denervation precedes microglial activation in cervical cord.

P234   TESTOSTERONE AND SYNTHETIC ANABOLIC STEROIDS ARE MODIFIERS FOR MUTANT SOD1-RELATED ALS PATHOGENESIS

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Keywords: androgens; androgen receptor; anabolic steroids

Background: Evidence indicates a higher incidence of sporadic ALS (sALS) in males than in females. This difference is very marked in juvenile forms of ALS, but it declines with age, when androgen levels in the serum decrease. Varsity athletes, such as Italian soccer and American football players, and military veterans show a higher risk of developing ALS. Endogenous androgens act on the androgen receptor (AR), and anabolic synthetic steroids, such as nandrolone decanoate, are androgenic compounds illegally used as performance-enhancing agents. This suggests a possible correlation between the risk of developing SALS and dysregulation of androgen signalling.

Objective: To verify this hypothesis, we tested the impact of androgen signalling in the pathogenesis of SOD1-related ALS in transgenic mice expressing mutant SOD1^{G93A}. Mice were either castrated to eliminate serum androgens or treated with nandrolone decanoate to mimic the condition occurring in doped athletes.

Results: Castration resulted in a significant decrease in body weight of SOD1^{G93A} mice, but it enhanced muscle strength and motor coordination in ALS-affected mice. Castration also resulted in delayed disease onset and increased survival, although there was no significant effect on disease progression. Castration was associated with decreased levels of expression of AR. On the other hand, treatment of intact mice with nandrolone did not affect the body weight of SOD1^{G93A} mice, but it resulted in significant improvement in muscle strength and motor coordination. Nandrolone treatment did not affect disease onset and progression, though it increased survival compared to that of control mice. Nandrolone treatment resulted in increased EDL weight, strength and specific force, which was associated with an increase in speed contraction. However, nandrolone effects were also associated with increased fatigue. Surprisingly, treatment of castrated mice with nandrolone deteriorated phenotype, and this was associated with increased AR aggregation. Altogether, these results indicate that androgen signalling plays a role in SOD1-related ALS pathogenesis.

P235   H63D HFE SHORTENS SURVIVAL AND ACCELERATES DISEASE PROGRESSION IN AN ALS MOUSE MODEL

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Keywords: SOD1/H67D, iron, oxidative stress

Background: There is increasing evidence suggesting an association between H63D HFE and amyotrophic lateral sclerosis (ALS). The presence of H63D HFE is associated with disease processes implicated in ALS such as iron accumulation, oxidative stress, increased glutamate release, and endoplasmic reticulum stress. Therefore, we hypothesize that H63D HFE increases the risk of ALS by promoting the convergence of disease processes implicated in ALS.

Objectives: To create a double transgenic mouse line that carries H67D HFE (homologous to H63D in humans) and the SOD1^{G93A} mutation to examine how H63D HFE influences ALS pathogenesis.

Methods: We crossed an ALS mouse model SOD1^{G93A} with H67D mice to generate double transgenic mice (SOD1/H67D). Disease onset was determined by monitoring the motor performance on a rotarod. End-stage was defined as the inability of the animal to right itself within 30 s after being placed on its side. Disease duration was the mean time from onset to end-stage. A grip strength meter was used to measure forelimb and hindlimb strength as markers for disease progression. We measured expression of proteins involved in iron homeostasis and oxidative stress to determine mechanisms by which H63D HFE contributes to ALS. In all experiments, SOD1/H67D mice were compared with SOD1^{G93A} and wild-type littermates.

Results: The SOD1/H67D mice have a shorter survival and more rapid rate of disease progression than SOD1^{G93A} mice, although age of disease onset is not different between two groups. Expression of the transferrin receptor (TfR) involved in cellular iron uptake is decreased in both SOD1/H67D and SOD1^{G93A} mice starting at 90 days. However, in SOD1/H67D mice, decreased TfR expression is accompanied by increased L-ferritin, an iron storage protein. Increased heme-oxygenase-1 and caspase-3 expressions are present at an earlier age in SOD1/H67D compared to SOD1^{G93A} mice. The elevated oxidative stress in SOD1/H67D mice may be the result of impaired cellular stress response by nuclear factor E2-related factor 2 (Nrf2), which is lower in SOD1/H67D than in SOD1^{G93A} mice.

Discussion and conclusion: It is noteworthy that no dietary or environmental challenges were presented to the double transgenic mice; therefore, our findings strongly indicate that H63D HFE is a genetic modulator of ALS. Mechanisms underlying more rapid disease progression observed in SOD1/H67D mice include disrupted iron homeostasis and oxidative stress. Because as many as 30% of ALS patients carry the H63D HFE polymorphism, our animal model has meaningful clinical implications and can serve as a preclinical model when evaluating the impact of H63D HFE on treatment strategies for ALS patients.

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P236 CHARACTERISING DISTAL DYSFUNCTION AND DEGENERATION IN ALS: THE POTENTIAL FOR AXON PROTECTION?

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Keywords: distal degeneration, neuromuscular junction, axon protection

Background: ALS is characterised by degeneration of the motor axons in the corticospinal tract and lower motor neurons. This die-back also involves the loss of acetylcholine receptor (AChR's) at the neuromuscular junction (NMJ). The precise time course of distal pathology development and NMJ dysfunction in ALS is yet to be fully elucidated. This information is critical for revealing novel drug targets and for specifically investigating the potential therapeutic strategy of 'distal protection' for improving ALS outcomes.

Objectives: To characterise the development of distal dysfunction and pathology development at both the axon and NMJ in mSOD1^{G93A} and YFPxmSOD1^{G93A} cross transgenic models, and characterise the recently developed novel model of site-specific excitotoxicity prior to determining the effect of treatment with the microtubule stabilizing agent Epothilone D on the development of disease phenotype.

Methods: YFP (Thy1) effects on motor phenotype were undertaken to account for transgene effects (n = 5) using rotarod performance test (TSE and USA), grip strength analysis (Chatillion, USA), and weight change over 11 weeks. Comprehensive immunohistochemical and confocal investigation of mSOD1^{G93A} (4, 8, 12, 16, and 20week) and YFP x mSOD1^{G93A}cross (8 and 20weeks) transgenic gastrocnemus muscles (n = 4/group, PFA-fixed, 40- or 80-μm frozen sections) was subsequently performed.

Results: Analysis of motor phenotype in YFP mice identified no significant change compared to that in wild type due to transgene expression. Immunohistochemical analysis showed that at 20-week-old YFP x mSOD1^{G93A} mice had a ratio of 1:1 degenerating axons to healthy axons (2.252 ± 0.92, 2.67 ± 1.01, respectively), with a global loss of axons also apparent. AchR staining in both the YFP x mSOD1^{G93A} cross, and the mSOD1^{G93A} tissue resulted in NMJs with fragmented morphology. Non-co-localized NMJs (αBt-YFP) increased from 8 to 20 weeks (2.15 ± 0.84, p < 0.001). Fully co-localized NMJs showed degenerative morphology at 8 weeks (1.31 ± 0.42, p < 0.05). A significant (p < 0.001) increase in the size of degenerating NMJs occurred over disease progression from 8 to 20 weeks. Between 8 and 20 weeks, NMJs positive for S100 decline (52.4 ± 3.4, 20.5 ± 12.8 SOD; 65.4 ± 4.8, 45.8 ± 6.7 WT); conversely, nestin immunoreactivity significantly (p < 0.05) increases at 8 weeks (73.3 ± 6.7 SOD; 5.0 ± 2.6 WT).

Discussion and conclusion: The changes in axonal pathology typical of ALS offer a key site for potential intervention for future therapeutics. Complete characterization of the functional and pathological changes occurring in the distal axon and NMJ in the mSOD1^{G93A} and our novel site specific excitotoxicity model will allow for subsequent testing of novel therapeutic targets directed at distal protection. Specifically, drugs such as the microtubule stabilizer Epothilone D may

allow improved microtubule function, resulting in sustained axonal transport and subsequently less degeneration and pathology.

P237 TARGETED ABLATION OF MYELINATING SCHWANN CELLS ENHANCES DISEASE SEVERITY IN SOD1G93A MICE

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Keywords: SOD1, Schwann cells, diptheria toxin

Background: Mutant SOD1 action within non-neuronal cells is implicated in damage to motor neurons in genetic forms of ALS. Astrocytes, microglia and oligodendrocytes drive progression in mutant SOD1 mice, while the role of Schwann cells is less clear. Schwann cells, the myelinating glial cells of the peripheral nervous system, are intimately associated with motor neurons and are vital for nerve conduction, axonal development, transport and support. The role of Schwann cells in ALS pathogenesis is unequivocal. Selective removal of mutant SOD1 from Schwann cells accelerates disease progression, while restricted expression of mutant SOD1 in Schwann cells is not harmful to mice. To resolve the contribution of Schwann cells to ALS, we generated double transgenic mutant SOD1; MBP-DTR mice that allows selective elimination of myelinating Schwann cells.

Objectives: To investigate the effect of myelinating Schwann cell depletion on disease onset, progression and spinal cord and peripheral nerve pathology in SOD1^{G93A} mice.

Methods: SOD1^{G93A} mice were crossed with novel transgenic MBP-DTR mice which express diphtheria toxin receptor (DTR) driven by the myelin basic protein (MBP) promoter. Exogenous administration of diphtheria toxin (DT) to MBP-DTR mice results in selective ablation of 25% of myelinating Schwann cells that is sublethal. Double transgenic SOD1^{G93A}MBP-DTR mice and control genotypes SOD1^{G93A}; MBP-DTR; and wild-type (WT) were injected with DT (10μg/kg, ip) at presymptomatic age (P60). Disease onset and progression was determined using rotarod and grid test performance, and survival was assessed. Spinal cords and sciatic nerves were analysed by immunohistochemistry and electron microscopy for motor neuron and axonal counts, Schwann cell apoptosis and myelination.

Results: Administration of DT provoked hindlimb weakness and muscle wasting in MBP-DTR mice, which peaked at 22 days post injection (P 82), followed by rapid recovery by 28 days post injection (P88). This resulted from 25% depletion of Schwann cells and demyelination in sciatic nerves. Double transgenic SOD1^{G93A}MBP-DTR mice showed increased severity of muscle weakness and wasting at peak symptoms and Schwann cell loss and demyelination. Administration of DT to SOD1^{G93A} or WT mice did not elicit symptoms or Schwann cell death as mice are naturally resistant to diphtheria.

Discussion and conclusion: Our data demonstrate that DT-induced myelinating Schwann cell ablation and resulting motor dysfunction is enhanced by mutant SOD1 expression in Schwann cells. This suggests that mutant SOD1 damage to Schwann cells sensitises them to death in this toxin-induced model.

P238   BLOCKING DEATH RECEPTOR 6 (DR6) PROMOTES NEUROMUSCULAR JUNCTION INTEGRITY AND FUNCTIONAL RECOVERY IN MOUSE MODEL FOR ALS

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Keywords: motor neuron protection, axon integrity, functional recovery

Background: Death receptor 6 (DR6) is a TNFR superfamily member with death domain selectively expressed in CNS. DR6 levels are elevated in spinal cords from postmortem samples of human ALS and from SOD1G93A transgenic mice.

Objective: To determine whether blocking DR6 by its antagonist antibody promotes motor neuron survival *in vitro* and in SOD1G93A mice.

Methods: Human and rat primary motor neuron cultures were used to determine whether blocking DR6 by anti-DR6 antagonist monoclonal antibody, 5D10 (1,3), protected motor neurons from death using three methods: growth factor removal; sodium arsenite to induce mitochondrial oxidative stress; and astrocyte (SOD1^{G93A})-induced cytotoxicity in motor neuron and astrocyte co-culture. We also investigated the neuroprotective effects of blocking DR6 in SOD1^{G93A} mice by using immunohistochemical staining to quantify the effects of anti-DR6 on neuromuscular junctions (NMJ), motor neurons in spinal cord, gliosis and plasma levels of phosphorylated neurofilament heavy chain (pNFH).

Results: Blocking DR6 with an antagonist antibody (5D10) promotes motor neuron survival *in vitro* resulting from growth factor withdrawal, sodium arsenite treatment, or co-culturing with SOD1^{G93A} astrocytes. When compared to control treated littermates, 5D10 treatment protects NMJs from denervation, increased numbers of motor neurons, decreased gliosis in the spinal cords, decreased plasma pNfH levels, and promotes functional recovery in the SOD1G93A mice.

Discussion and conclusion: DR6 has emerged as an important regulator of oligodendrocyte and neuronal cell death (1–3). DR6 antagonism can protect motor neuron survival *in vitro* and in SOD1^{G93A} mice. Blocking DR6 function may represent a new approach for the treatment of neurodegenerative disorders involving motor neuron death and axon degeneration such as ALS.

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P239   EXCITOTOXICITY AND NEUROMUSCULAR JUNCTION DEGENERATION FOLLOWING SITE-SPECIFIC EXCITOTOXIN EXPOSURE *IN VIVO*

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Keywords: excitotoxicity in vivo mouse

Background: Amyotrophic lateral sclerosis (ALS) is likely to be a multifactorial disease of neuronal dysfunction and loss; however, recent investigations indicate that axonal dysfunction, prior to cell loss, may be the causative factor of the initial symptoms of ALS and that distal axonal degeneration may occur before the onset of disease symptoms.

Purpose: Our investigations are focused on determining the degenerative changes underlying ALS-like axonopathy using site-specific excitotoxic insults, via osmotic mini pumps, to the spinal cord and muscle.

Methods: To achieve site-specific excitotoxicity osmotic mini pumps (Alzet, model 1004), a constant chronic infusion to either the L3–4 lumbar region of the spinal cord or the gastrocnemius muscle in the hind limb was delivered. A constant and chronic infusion of Kanic acid (KA, 5–10mM, in cortex buffer with 1μM Fluro Ruby) was delivered to the subarachnoid space of the lumbar region (L3–5) of C57/Bl6 mice and transgenic mice which express yellow fluorescent protein (YFP) in a subset of motor neurons on a C57/BL6 background. At the gastrocnemius muscle, glutamate (5–10mM in saline with 1μM Fluro Ruby) was chronically infused in YFP mice. Animals were perfused at a range of time points, and the degree of axonal degeneration was investigated through immunohistochemistry and confocal microscopy.

Results: Fluoro ruby labelling was present throughout cells within the subarachnoid space in L3–L5 and muscle fibres of the gastrocnemius muscle, indicating that a targeted delivery can be achieved with the osmotic pumps. Quantitation of the number of neurons (cell body > 20 μm) stained with toluidine blue within the anterior ventral horn at 7, 14 and 28 days post surgery (DPS) demonstrated a significant (p < 0.05) decrease in number of motor neurons at 28 DPS in the 5 mM- and 10 mM KA-treated mice in comparison with vehicle control. Gastrocnemius muscles of the KA and vehicle control spinal cord treated mice were double labelled with synaptophysin and alpha-bunglarotoxin to determine the amount of neuromuscular junction (NMJ) degeneration. Synapses were graded as either intact or degenerating. There was a significant (p < 0.05) increase in the percentage of degenerating synapses in the KA mice in comparison with that of control. Excitotoxin application to the gastrocnemius muscle (5–10 mM glutamate) resulted in a localised synapse loss. However, the cell bodies in the spinal cord did not undergo degeneration over the 28 DPS time course.

Conclusion: Our results indicate that motor neuron degeneration is dependent upon the site of exposure to excitotoxin. Identifying the site of the initial effects of excitotoxicity will identify mechanisms of distal axon degeneration that may provide novel therapeutic targets directed at axon protection.

P240   IDENTIFICATION OF NOVEL THERAPEUTICS TO TREAT NEURODEGENERATION USING THE SOD1 ZEBRAFISH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: zebrafish, therapeutic, screening

Background: We have utilised a mutant SOD1 zebrafish model containing the mutant *sod1* gene in tandem with stress readout (HSP70-DsRed) reporter gene as a measure of neuronal stress. Previously it has been shown that our model exhibits neuronal stress in the spinal inhibitory interneurons that eventually transfers to stress and results in functional changes in the motor neurons and neuromuscular synapse formation. We have also previously shown that using riluzole, currently the only approved treatment for ALS, we can reduce neuronal stress in our model.

Objectives: To utilise this zebrafish model and to develop and perform a high-throughput compound screen to identify compounds that reduce SOD1-mediated neuronal toxicity. The secondary aim is to design and optimise secondary screens to further refine our key hit compounds and to identify potential future clinical therapeutics.

Methods: Until 6 days post fertilisation (dpf) by immersion, 48hpf sod1 transgenic zebrafish were treated with compounds obtained through the Microsource spectrum library . At 6dpf, the larvae were imaged, sonicated and total fluorescence measured using a fluorescence plate reader. Strictly standardised mean difference (SSMD) was used to select hit compounds (β-value below −0.5 were considered as hits in this assay). Dose responses, adult compound dosing, seahorse mitochondrial analysis, and imaging were used as secondary screens.

Results: A high-throughput drug screen has been developed with the capability to screen over 100's of compounds per day with an assay specificity and sensitivity of approaching 100%. We have screened 2000 compounds from the spectrum library in triplicate and have identified 61 different modulators of neuronal stress. From this screen, we have identified 42/2000 compounds which show reduced neuronal stress and 19/2000 which induced a strong hsp70 activation, a cellular repair pathway. Using secondary screens, we have identified a selection of hit compounds that have a positive role in reducing neuronal stress in mutant SOD1 zebrafish. In conclusion, we have designed and validated a drug screen capable of screening 1000's of compounds rapidly and accurately. Using this screen we have identified compounds with a positive role in reducing neuronal stress which are being taken further developed as potential new treatments for human disease.

P241   CHEMICAL GENETIC SCREENS OF TARDBP AND FUS MODIFIERS IN C. ELEGANS AND ZEBRAFISH

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Keywords: drug discovery, TDP-43, FUS

Background: Two recently discovered causative genes for ALS are the *TARDBP* gene (coding for TDP-43 and also mutated in rare cases of FTLD) and *FUS/TLS*. Since TDP-43 and FUS are evolutionarily conserved, we turned to the model organisms *Caenorhabditis elegans* and zebrafish to learn more about their biological functions and screen for potential therapeutic modifiers.

Methods: Transgenic expression of wild-type and mutant human *TARDBP* (1) and *FUS* (2) was obtained upon mRNA injection into zebrafish blastulae. We obtained deletion mutants for the worm orthologues *tdp-1* and *fus-1* and engineered strains to express human *TARDBP* or *FUS* in worm motor neurons (3). We then screened 3,700 FDA-approved compounds in the Microsource Discovery Spectrum, Biomol, Prestwick and Sigma libraries using mutant *TARDBP* worms and validated hits in zebrafish and in mutant FUS lines.

Results: We performed motility assays in zebrafish and lifespan and stress response assays in worms. We observed that *TARDBP* and *FUS* have roles in the response to oxidative and osmotic stress. The expression of mutant TDP-43 or FUS in worm motor neurons produces robust, adult onset motility defects, and in both models this was caused by motor neuron deficits. We isolated a number of chemical suppressors of mutant *TARDBP* toxicity.

Discussion: Under normal conditions, TDP-43 and FUS regulate specific aspects of the cellular stress response. The transgenic models allowed us to isolate chemical suppressors of motor defects. In particular, several neuroleptics protected against development of the motor phenotype and one is currently in clinical trial.

Conclusion: Together these data provide clues to help unravel the mechanism for TDP-43 and FUS toxicity that should also provide leads for early drug discovery.

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P242   IDENTIFICATION OF RNA BOUND TO TDP-43 SUPPORTS ITS ROLE IN SYNAPTIC FUNCTION

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Keywords: TDP-43, RNA-binding protein, C. elegans

Background: Transactive response DNA-binding (TDP-43) protein is an RNA binding molecule that is known to regulate activities including transcription and pre-mRNA splicing. The importance of TDP-43 in ALS pathogenesis has been highlighted by its presence in cytoplasmic inclusions in the central nervous system (CNS) of ALS patients, as well as by the discovery of mutations in *TARDBP* (the gene encoding TDP-43). The normal role of TDP-43 in the CNS is yet to be fully determined, and how mutations lead to neurodegeneration remains unknown.

Objectives: The aim of the present study was to identify RNA targets of TDP-43 in the mammalian CNS, and to generate a *Caenorhabditis elegans* model to study molecular pathways that are involved in TDP-43-mediated neurodegeneration.

Methods and results: RNA immunoprecipitation followed by microarray analysis (RIP-chip) was used to identify RNAs bound to TDP-43 in the mouse brain. The RIP-chip analysis produced a list of 1839 potential target genes many of which overlap with previous studies, and whose functions include RNA processing and synaptic function. We have also shown by immunohistochemistry that TDP-43 is localized at the mouse neuromuscular junction. We next generated a *C. elegans* transgenic strain in which human TDP-43 (normal TDP-43 as well as the A315T and M337V ALS causing variants) was selectively expressed in the GABAergic motor neurons. Worms expressing mutant TDP-43 show disrupted presynaptic loci, prior to the onset of changes to neuronal morphology. Using a GFP-tagged version of TDP-43, we investigated the intracellular localization and found that TDP-43 was predominantly nuclear localized, but was also seen in axons and puncta consistent with synaptic loci.

Discussion and conclusion: We are characterizing both mouse and *C. elegans* models to study the function of TDP-43 in the CNS. Our results support a role of TDP-43 in the transport of synaptic mRNAs down the axon to synapses for local translation. Disrupted axonal mRNA transport to distal processes may be a mechanism that leads to motor neuron death in ALS.

P243   TRANSLATIONAL PROFILING IN TDP-43 TRANSGENIC MOUSE MODEL OF ALS

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Keywords: translational profiling, microarray, immunofluorescence

Background: A major feature of degenerating motor neurons in ALS is the mislocalization of the transactive response DNA-binding protein of 43kDa (TDP-43) from the nucleus to the cytoplasm, forming ubiquitinated inclusions. Mutations in TDP-43 account for a small portion of ALS cases; however, TDP-43 pathology is observed in over 90% of cases, indicating that abnormalities in TDP-43 are an important contributor to disease pathogenesis.

Objective: Since TDP-43 is a nuclear DNA- and RNA-binding protein that has known functions in regulating RNA metabolism, it is likely that abnormalities in TDP-43 will be reflected in changes in RNA processing and expression. Our objective is to identify these changes as a means to understanding how abnormal TDP-43 contributes to ALS pathogenesis.

Methods: Typical approaches to identifying changes in RNA expression (transcriptional profiles) rely on analyzing total mRNA pools from a tissue region or specific cell type. We used a novel technique entitled translating ribosome affinity purification (TRAP) to identify mRNAs actively being translated from spinal cord motor neurons of TDP-43[A315T] mice. Bacterial artificial chromosome transgenic mice expressing an EGFP-tagged ribosomal protein, L10a, under the control of the choline acetyltransferase promoter were crossed with TDP-43[A315T] mice to facilitate affinity purification of translating mRNAs from motor neuron polysomes. Translational profiles were obtained using microarray analysis in symptomatic (10 months old) TDP-43[A315T] mice and were compared to wild-type (WT) littermates. Unpaired two-tailed t-test with Benjamini–Hochberg correction was used to determine significantly varying probes between TDP-43[A315T] and WT. The Biological Networks Gene Ontology Tool (BiNGO) plugin in Cytoscape was used to identify overrepresented GO terms. Genes with a $\geq$ 2-fold change between TDP-43[A315T] and WT were validated using immunofluorescence.

Results: Symptomatic translational profiles showed that 28 genes were significantly misregulated. BiNGO analysis demonstrated that there was overrepresentation of genes involved in RNA metabolic process (GO-ID 0016070, p = 3.27E-02), immune response (GO-ID 0006955, p = 3.39E-02), and regulation of mitotic recombination (GO-ID 0000019, p = 3.27E-02) in the TDP-43A315T mice. Of the 28 misregulated genes, 20 are mapped and 7 had a fold change of $\geq$ 2. Validation with immunofluorescence was representative of microarray results.

Discussion and conclusion: Symptomatic TDP-43[A315T] mice showed misregulation of genes enriched for RNA processing, immune response, and cell cycle regulation, all of

which are highly implicated in ALS pathogenesis and other neurodegenerative diseases. Immunofluorescence in TDP-43[A315T] motor neurons was indicative of microarray and will be validated in patient samples. This discovery-based approach has, for the first time revealed translational changes in motor neurons of a TDP-43 mouse model and will provide a greater understanding of the mechanistic basis of motor neuron degeneration.

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P244   SENATAXIN MOUSE MODELS OF ALS4 RECAPITULATE HUMAN ALS TDP-43 PATHOLOGY, DEVELOP NEUROMUSCULAR PHENOTYPES, AND EXHIBIT TRANSCRIPTIONAL ALTERATIONS RESULTING IN DEMYELINATION

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Keywords: SETX, TDP-43 mislocalization, myelin

Background: Autosomal dominant, gain-of-function mutations in the senataxin (*SETX*) gene cause a juvenile onset form of amyotrophic lateral sclerosis, known as ALS4. To determine the mechanistic basis of ALS4 motor neuron degeneration, we derived two different mouse models carrying human ALS4 mutations.

Methods: In this study transgenic lines expressing wild-type senataxin and the R2136H mutation in murine prion protein promoter expression constructs (PrP-SETX[wt] and PrP-SETX[R2136H]), and a knock-in line containing the L389S substitution mutation at the mouse senataxin locus (SETX-[L389S]-KI) were generated. Characterization of these mice was performed using composite phenotype scoring, and by evaluating motor function through rotarod testing and stride length measurements.

Results: This analysis revealed that both mutant mouse lines develop a slowly progressive motor phenotype, with impaired rotarod performance, presence of hind limb clasping, and ledge test abnormalities. When we immunostained lumbar spinal cord sections from *SETX* mutant mice, we observed nuclear clearing of TDP-43, accompanied by TDP-43 cytosolic aggregation, which was indistinguishable from the hallmark pathology observed in human ALS patients.

To elucidate the molecular basis of senataxin gain-of-function pathology in the spinal cord, we isolated RNA from ventral horn samples obtained from sets of early symptomatic PrP-SETX-[R2136H] and SETX-[L389S]-KI mice, and performed RNA-Seq to catalogue transcriptome changes associated with the ALS4 disease state. We found that PrP-SETX-[R2136H] mice exhibited significant expression alterations in 188 spinal cord-expressed genes, and SETX-[L389S]-KI

mice displayed significant expression changes in 304 spinal cord-expressed genes, with coordinate overlapping gene expression alterations detected for 71 genes. Analysis of the shared gene set subject to alteration revealed a preponderance of genes implicated in the myelination pathway: *Egr2*; *Pmp22*; *Prx*; and *Mpz* mutated in Charcot-Marie-Tooth disease. When we re-examined autopsy material from human ALS4 cases, we noted loss of myelin in peripheral nerves, ventral horn, and dorsal horn regions.

Discussion: These findings are consistent with evidence from sporadic ALS for myelin loss, suggesting that these ALS4 mouse models have uncovered a previously unappreciated aspect of ALS disease pathogenesis in human patients.

P245   A BAC-BASED MOUSE MODEL OF TDP-43-ASSOCIATED ALS AS A TOOL TO EXPLORE EARLY-PHASE PATHOGENESIS

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Keywords: TDP-43, bacterial artificial chromosome, mouse model

Background: Mutations in TAR DNA-binding protein 43 (TDP-43) are associated with ALS, though the molecular mechanisms leading to pathogenesis are still unclear. TDP-43 becomes mis-localised to the cytoplasm of affected motor neurons in most sporadic ALS patients, where it forms characteristic insoluble, ubiquitinated inclusions. TDP-43 cDNA overexpression models driven by ubiquitous or neuronally expressed promoters display highly variable disease phenotypes.

Objective: To more accurately model ALS, we have generated transgenic mice using bacterial artificial chromosome (BAC) constructs expressing human wild-type (WT) or ALS-associated mutant (M337V) TDP-43 under the control of the endogenous human promoter.

Methods: BAC vectors containing the full-length human genomic locus of the WT or M337V mutation, with an Ypet tag, were targeted to the ROSA26 locus in embryonic stem cells (ESCs) by PhiC31 integrase-mediated cassette exchange. Chimeric mice were generated by blastocyst injection of recombinant ESCs which were subsequently crossed with C57BL/6J female mice to generate two isogenic human TDP-43 transgenic lines, differing only by the presence or absence of the M337V mutation.

Results: We have confirmed that a single BAC copy of the TDP-43-WT-Ypet and TDP-43-[M337V]-Ypet is sufficient to drive physiologically relevant levels of transgene expression by immunoblotting. By 2 months of age, elevated levels of human TDP-43 and total TDP-43 are observed in insoluble brain and spinal cord protein fractions from homozygous TDP-43[M337V]-Ypet mice compared to TDP-43-WT-Ypet and non-transgenic littermate controls. Accompanying this is a decrease in human TDP-43 mRNA levels in the spinal

cord of TDP-43[M337V]-Ypet mice compared to TDP-43-WT-Ypet mice, which is not observed in the brain. Expression of endogenous mouse TDP-43 mRNA is unchanged in brain and spinal cord of both transgenic lines compared to non-transgenic controls. Immunostaining of brain and spinal cord sections with antibodies directed against total TDP-43, human-specific TDP-43, and GFP confirm the ubiquitous expression of TDP-43 throughout the CNS, and specifically the expression of human TDP-43 in brain and spinal cord of the transgenic lines.

Discussion and conclusion: BAC transgenic mice represent the state-of-the-art in transgenic mouse models, since transgenes are expressed at physiological levels from native promoter regions. Parallel cohorts of non-transgenic, heterozygous and homozygous mice from each line are being monitored with an array of behavioural, biochemical and neuropathological testing to study the emergence of phenotypic changes. In combination with longitudinal RNA splicing analysis timed to compare pre-symptomatic with various stages of symptomatic mice, we will address the fundamental role of TDP-43 mutation in ALS. Elucidation of early pre-symptomatic changes in cellular homeostasis has the potential to allow the identification of biomarkers for human ALS and to identify the most therapeutically tractable targets for drug development.

P246   ABERRANT PERIPHERIN EXPRESSION AND STABILITY AND SPLICING OF GLT-1 IN TDP-43 (A315T) TRANSGENIC MOUSE MODEL OF ALS

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Background: TDP-43 is an RNA/DNA-binding protein implicated in ALS. Cytoplasmic TDP-43-positive inclusions are found in motor neurons of patients with ALS suggesting a potential loss of function (1). The function of TDP-43 in the nucleus is to regulate transcription and RNA metabolism, including RNA splicing and editing. Aberrant RNA splicing of peripherin and EAAT2 (excitatory amino acid glutamate transporter 2) have been implicated in ALS (2,3).

Objective: We investigated whether TDP-43 regulates RNA splicing of peripherin and GLT-1, the murine homologue of EAAT2, in the TDP-43[A315T] transgenic mouse model of ALS. We also examined peripherin expression and stability in TDP-43[A315T] and SOD1[G93A] transgenic mice.

Methods: To identify novel splice variants, we designed primers that amplify the entire coding sequence of both peripherin and GLT-1 and also Per-61-specific primers. We performed sequential extraction of neurofilaments from lumbar spinal cords (LSCs) using low- and TX-100-containing high salt buffer to determine peripherin expression and stability in both TDP-43[A315T] and SOD1[G93A]. We also performed Western blot to check the protein levels of both the glial-specific GLT-1 and the neuronal isoform GLT-1B in TDP-43[A315T].

Results: We found that Per-61 was expressed in all genotypes including the wild-type mice at both RNA and protein levels. No novel alternative splice variants were found in peripherin

nor GLT-1. In symptomatic mice, Per-45 translation was selectively up-regulated in TDP-43[A315T]. Moreover, treating neurofilaments with Triton X-100 resulted in significant increase in soluble Per-56 and Per-45 in samples isolated from TDP-43[A315T] mice (*P*-values: 0.02 and 0.003, respectively). Conversely, soluble Per-45 and Per-56 levels were reduced significantly (*P*-values: 0.009 and 0.03, respectively) in SOD1[G93A] mice. In contrast, no change was detected in the Triton-insoluble fractions of both genotypes. In addition, the relative expression of GLT-1B was significantly downregulated in both symptomatic and non-symptomatic TDP-43[A315T] mice (*P*-values: 0.03 and 0.004, respectively).

Discussion and conclusion: Our data suggest that overexpression of TDP-43[A315T] induces upregulation of Per-45 translation and leads to the instability of the filament network. Conversely, overexpression of SOD1[G93A] enhances neurofilament stability. Moreover, the reduction of GLT-1B expression in TDP-43[A315T] mice suggests impaired splicing and therefore might exacerbate disease progression.

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P247   MUTANT TDP-43 DEREGULATES THE AMPK SIGNALLING CASCADE THROUGH NOVEL ACTIVATION OF PROTEIN PHOSPHATASE 2A (PP2A)

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Keywords: AMPK, PP2A, TDP-43

Background: Hypermetabolism and defective energy homeostasis are implicated in motor neuron degeneration in ALS patients and mutant SOD1 mouse models. However, whether energy hypermetabolism occurs in mutant TDP-43-mediated ALS remains unclear. Here, we investigated activation of the AMP-activated protein kinase (AMPK) signalling cascade and its regulation by upstream kinases and downstream phosphatases in models expressing mutant TDP-43 linked to ALS.

Objectives: To examine AMPK signalling and its regulation by kinases and phosphatases in cell culture and mouse models expressing mutant TDP-43.

Methods: Activation of the AMPK signalling pathway was examined using Western blotting and immunocytochemistry for phosphorylated AMPK and downstream target acetyl-CoA carboxylase (ACC) in NSC-34 cells stably transfected with wild-type (WT) or mutant TDP-43. AMPK signalling was also examined in brains and spinal cords of presymptomatic (P30, 60) and symptomatic (P90) transgenic TDP-43[A315T] mice and compared to age-matched WT controls.

AMPK regulation was examined in stable cells and mice using Western blotting for kinases (CAMKII and LKB1) and phosphatases (PP2A, PP2Cand PP1-R6).

Results: AMPK activity was drastically diminished by 80% and 60% in spinal cords and brains of transgenic TDP-43[A315T] mice at pre-symptomatic and symptomatic disease, respectively. AMPK activation was also reduced ~50% in NSC-34 cells expressing different TDP-43 mutants. To determine how mutant TDP-43 deregulates AMPK signalling, we screened AMPK kinases and phosphatases, revealing that PP2A level was sharply increased in tissues of mice and NSC-34 cells expressing mutant TDP-43. Furthermore, treatment of NSC-34 cells with the AMPK agonist AICAR did not normalise AMPK activity level, consistent with PP2A-dependent regulation.

Discussion and conclusion: In contrast to mutant SOD1 models, we show that energy homeostasis mediated by the AMPK signalling cascade is severely deregulated in both pre-symptomatic mice and cell cultures expressing mutant TDP-43. We also uncover a novel regulation of PP2A activity by mutant TDP-43. Thus, overstimulation of PP2A signalling in motor neurons by mutant TDP-43 presents a new potential player in ALS pathogenesis.

P248　ADAR2 PLAYS A KEY ROLE FOR DEATH AND TDP-43 MISLOCALIZATION IN ALS MOTOR NEURONS

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Keywords: TDP-43, AMPA receptor, gene therapy

Background: Failure of GluA2 RNA editing resulting from downregulation of the RNA-editing enzyme adenosine deaminase acting on RNA 2 (ADAR2) occurs in the majority of ALS cases and causes death of motor neurons via a Ca^{2+}-permeable AMPA receptor-mediated mechanism. Notably, downregulation of ADAR2 is associated with TDP-43 mislocalization in the motor neurons of conditional ADAR2 knockout (AR2) mice that mimic molecular changes in sporadic ALS motor neurons, but the underlying molecular mechanism remained to be elucidated.

Methods: We found that the expression of Q/R site-unedited GluA2 and resultant upregulation of Ca^{2+} permeable AMPA receptors upregulated calpain, a Ca^{2+}-dependent serine protease, via increasing cytoplasmic Ca^{2+}. Calpain cleaved TDP-43 in the C-terminal region and generated aggregation-prone N-terminal fragments, which served as seeds for cytoplasmic inclusions. It is likely that full-length TDP-43 that shuttles between the nucleus and the cytoplasm sequestrates into the inclusion, which results in the elimination of TDP-43 from the nucleus, thereby forming TDP-43 pathology. Calpain sequentially cleaved TDP-43 into soluble smaller fragments, indicating that only an optimum level of calpain activation allowed formation of aggregates. Both TDP-43 mislocalization and neuronal death were inhibited when the Ca^{2+} influx though the AMPA receptors were normalized.

Results: These lines of evidence prompted us to develop gene therapy for sporadic ALS by means of global delivery of the *ADAR2* gene to motor neurons. AAV9-*ADAR2* injected in the tail vein of AR2 mice caused effective expression of *ADAR2* in the motor neurons, and motor dysfunction and neuronal death were prevented in pre- and post-symptomatic AR2 mice. Notably, TDP-43 mislocalization in the motor neurons was normalized in the remaining motor neurons. Therefore, delivery of *ADAR2* using AAV9 as a vector enabled restoration of RNA-editing activity at the GluA2 Q/R site, which provides mechanistic therapeutic strategy for patients with sporadic ALS.

Discussion: In conclusion, ADAR2 downregulation induces death of motor neurons via Ca^{2+}-permeable AMPA receptor-mediated mechanism and causes mislocalization of TDP-43 via activating calpain-mediated cleavage. Delivery of the ADAR2 gene globally to motor neurons effectively rescued motor neurons with normalization of TDP-43 localization in sporadic ALS model mice.

P249　ULTRASTRUCTURAL CHANGES IN THE BLOOD–SPINAL CORD BARRIER IN TDP-43 CONDITIONAL KNOCKOUT MICE

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Keywords: TDP-43 knockout mice, blood-spinal cord barrier, ultrastructure

Background: TDP-43 conditional knockout (TDP CKO) mice develop progressive weight loss and motor impairment around the age of 60 weeks, and degeneration of large anterior horn neurons at the age of 100 weeks. Although the loss-of-function of TDP-43 protein seems to affect the pathogenesis of ALS, the interaction between the loss of TDP-43 protein and the changes in the blood–spinal cord barrier (BSCB) remains unclear.

Objectives: To clarify whether the loss of TDP-43 protein causes changes in the BSCB in TDP CKO mice.

Methods: We studied lumbar spinal cords in eight TDP CKO mice and eight control littermates using electron-microscopy. TDP CKO mice were divided into four groups: early pre-symptomatic (aged 20 weeks); late presymptomatic (36 weeks); early symptomatic (50 weeks); and late symptomatic (100 weeks) stages (n = 2, respectively). TDP-43[flox/flox] mice served as age-matched control littermates in each group (n = 2).

Results: In controls, endothelial cells of the capillaries were characterized by tight junctions and some cytoplasmic organelles such as mitochondria, Golgi apparatus, rough endoplasmic reticulum, free ribosomes, vesicles and multivesicular bodies. The perivascular space of the capillaries was bordered on one side by the basal lamina of the endothelial cell and on the other by the basal lamina of the astrocytic foot processes. Sometimes, the perivascular space was virtually

obliterated and the two laminae formed a single homogenous structure. In the anterior horn, the majority of the capillaries were surrounded by a narrow perivascular space without connective tissue. Pericytes were found outside the endothelial cells, and completely surrounded by a basal lamina.

In TDP CKO mice, the capillaries at the early presymptomatic, late presymptomatic, and late symptomatic stages were well preserved as in age-matched controls. At the early symptomatic stage, tremor appeared as the earliest symptom of motor deficit and the capillaries showed various alterations. In the anterior horn, the cytoplasm of most endothelial cells was severely vacuolated. The endothelium occasionally exhibited marked edema, detachment of the cytoplasm, and protrusion of the cytoplasm into the lumen of the blood vessel, although the tight junction appeared relatively intact. Perivascular spaces of the capillaries were frequently edematous. Mitochondria in the cytoplasm of endothelial cells and pericytes, and in perivascular spaces were frequently swollen and vacuolated. In the posterior horn, the structure of the capillaries was almost always normal.

Conclusions: The temporary and reversible breakdown of the BSCB with leakage or increased permeability at the early symptomatic stage could be a direct consequence of the loss of TDP-43 protein in the endothelial cells. The damage by the loss-of-function of TDP-43 protein to the vasculature contributes to initiate non-cell autonomous pathogenesis of degeneration of motor neurons in the mouse model observed.

P250   THE EFFECT OF CLUSTERIN ON THE TOXICITY OF GLIAL TDP-43 IN A DROSOPHILA MODEL OF ALS

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Keywords: TDP-43, clusterin, Drosophila

Background: Amyotrophic lateral sclerosis (ALS) is characterised by the presence of intracellular protein inclusions within degenerating neurons, and astro and microgliosis. Protein aggregation is implied as an underlying cause of disease pathology and recent studies suggest that non-neuronal cells play important roles in neurodegeneration through non-cell autonomous mechanisms (1,2). Clusterin is a conserved glycoprotein which under normal conditions is secreted from the endoplasmic reticulum (ER) into the extracellular space, where it acts as an extracellular chaperone. Recently, it has been shown that under conditions of ER stress, clusterin can be retro-translocated to the cytosol where it may act as an intracellular chaperone (3).

Objectives: To determine whether expression of TAR DNA-binding protein 43 (TDP-43; a major component of inclusions in ALS) in the glia of *Drosophila* is pathogenic, and whether co-expression of clusterin reduces this toxicity.

Methods: TDP-43 was expressed in the glial cells of *Drosophila* by crossing female TDP-43 flies with the male driver stocks eaat-1-GAL4 or eaat-1-GFP. The brains of larvae were dissected and stained for TDP-43 and neuronal markers.

Survival assays were performed on adult *Drosophila* expressing TDP-43, TDP-43 + Clusterin or clusterin in glial cells, as well as on 51D (non-transgenic) flies. Females of these stocks were crossed with the male driver line eeat-GFP-Gal80. A Kaplan–Meier survival curve was generated and differences between genotypes analysed using Kaplan Meir statistics.

Results: Expression of TDP-43 in larval glial cells resulted in a reduction of larvae size, locomotor defects and decreased hatching. Immunohistochemistry revealed motor neuron degeneration and suggested that TDP-43 was able to spread from glia to other cell types, including motor neurons.

Expression of TDP-43 in the glia of adult *Drosophila* was also toxic, with flies having a reduced lifespan (~12 days) when compared to that of the control flies (~30 + days). Flies that co-expressed clusterin had a significantly (p value < 0.0001) extended lifespan (~14 days).

Discussion and conclusion: These results support the theory that non-neuronal cells play important roles in neurodegeneration. The ability of clusterin to rescue TDP-43 toxicity suggests that it may play a role in the pathogenesis of neurodegenerative diseases in which protein aggregation underlies disease pathology. The pathogenic mechanisms of ALS are multifactorial, and it is likely that effective treatments can only be developed once these mechanisms are better understood. Potential therapeutic targets may eventually include drugs targeting glial cells and the use of chemical or peptide chaperones.

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P251   INVESTIGATING PROPAGATION OF TDP-43 AGGREGATION IN AMYOTROPHIC LATERAL SCLEROSIS USING A DROSOPHILA MELANOGASTER MODEL

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Keywords: TDP-43, Drosophila, propagation

Background: Symptoms of amyotrophic lateral sclerosis (ALS) characteristically have a focal onset and spread directionally and progressively throughout the central nervous system (CNS) (1). While the directional advancement of ALS pathology is well characterised, the molecular mechanisms underlying this process remain unknown. TDP-43, one of the major components of ubiquitinated inclusions associated with this disease, forms mislocalised aggregates capable of seeding aggregation of natively folded protein (2). Cell-to-cell transmission of misfolding proteins has been demonstrated for a number of neurodegenerative diseases, but little work has been carried out to investigate whether TDP-43 aggregation

initiated at a focal site, propagates throughout the CNS (3). Finding evidence for this proposed molecular model of ALS pathogenesis would explain how the disease progresses over time, and reveal new therapeutic targets to treat this debilitating condition.

Objectives: The objective was to develop a *Drosophila melanogaster* (fruit fly) model to investigate propagation of TDP-43 aggregates within the CNS to determine whether cell-to-cell transmission of the protein takes place.

Methods: TDP-43 expression was directed to a subset of photoreceptor neurons in the adult fly visual system using the Gal4-UAS system. Brains of 3-day-old adult flies were dissected and the distribution of TDP-43 analysed using immunohistochemistry and confocal microscopy.

Results: Transgenic TDP-43 driven in a subset of photoreceptors was localised in both the nuclei and the cytoplasm of these neurons, including the axonal projections targeting the optic lobes. TDP-43 also formed predominantly cytoplasmic puncta in cell populations beyond the Gal4 driver expression pattern when compared to controls.

Discussion and conclusion: Spreading of TDP-43 in the CNS was successfully modelled using the fly visual system. The results of this project show that TDP-43 can spread from a subset of neurons to other cell populations in the CNS, demonstrating that TDP-43 is transmissible between cells. Furthermore, TDP-43 forms cytoplasmic aggregates in recipient cells, recapitulating characteristic features of TDP-43-associated ALS pathology: nuclear mislocalisation and formation of aggregates. These findings support the proposal that TDP-43 aggregation can be propagated within the CNS from a focal site of onset, thus providing an explanation for how spatiotemporal spreading of ALS pathology occurs.

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P252   IDENTIFYING THE ROLE OF TDP-43 IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) THROUGH INTERACTOME ANALYSIS OF PATHOGENIC TDP-43 IN A TRANSGENIC MOUSE MODEL

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Keywords: interactome, alternative splicing, TDP-43

Background: TAR DNA-binding protein 43 (TDP-43) has been identified as a major protein in pathological inclusions of ALS and frontotemporal lobar degeneration (FTLD). A biochemical signature of TDP-43 proteinopathy is the presence of lower molecular weight (LMW) TDP-43 fragments. In understanding the origin of these LMW TDP-43 species, we have identified an abnormal splice variant of TDP-43 migrating at 35kD, herein referred to as TDP-35. TDP-35 expression is elevated in ALS tissues, and overexpression of TDP-35 in cell culture induces aggregate formation and cellular toxicity.

Objectives: To identify the role of TDP-35 in the pathogenesis of ALS, we have characterized transgenic mice overexpressing TDP-35 and performed interactome analysis of TDP-35 in order to elucidate disease-associated cellular pathways.

Method: Transgenic mice overexpressing human TDP-35 under the hamster prion promoter were characterized using protein biochemistry, immunohistochemistry, and motor and cognitive function tests. Interactome analysis was performed by perfusion crosslinking of animals, co-immunoprecipitation of TDP-35 complexes from brain homogenate, and use of mass spectrometry with iTRAQ labelling to identify proteins that co-purify with TDP-35. Candidates were validated using reciprocal IP and immunohistochemistry.

Results: Human TDP-35 is overexpressed in the brain and to a lesser extent in the spinal cord of transgenic animals. Younger transgenic mice exhibit a predominantly nuclear localization of TDP-35 while older mice show rare cytoplasmic inclusions and increased gliosis. No axonal loss, weight change, or overt motor phenotype is observed. Novel object recognition test revealed that at 11 months of age, but not at 6 months of age, transgenic animals exhibit significantly lower memory score than non-transgenic animals. Interactome analysis of TDP-35 in 12-month-old mice reveals several potential candidates. One of which, a protein involved in the transport of an excitatory neurotransmitter, has been validated by reciprocal IP, while the remaining are undergoing validation.

Discussion and conclusion: Mice overexpressing TDP-35 exhibit progressive cognitive dysfunction accompanied by increased gliosis in the brain. The lack of motor dysfunction and presence of cognitive phenotype may be attributed to the preferred expression of the hamster prion promoter in the brain versus the spinal cord. Given the presence of TDP-43 proteinopathy in FTLD and cognitive symptoms in a subset of ALS patients, TDP-35 may play a role in the cognitive aspect of these neurodegenerative diseases.

In conclusion, overexpression of TDP-35, an abnormal splice variant of TDP-43, is associated with cognitive dysfunction in mice and may underlie cognitive phenotypes in ALS and FTLD. The disease mechanism may involve abnormal transport of an excitatory neurotransmitter. Current investigation into TDP-35 interactome will shed light on the cellular pathways involved in neurodegeneration and provide additional insight into the role of excitotoxicity in ALS pathogenesis.

THEME 10 *IN VITRO* EXPERIMENTAL MODELS

THEME 10 *IN VITRO* EXPERIMENTAL MODELS

P253   BIOPHYSICAL AND BIOLOGICAL CHARACTERISATION OF INCLUSION BODIES CONTAINING TDP-43

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Keywords: amyloid, TDP-43, toxicity

Background: At present, it is not clear if TDP-43 intracellular inclusions accumulating in the cortex or spinal cord of patients are amyloid-like fibrils or another type of protein aggregates. Indeed, reports describing a structural characterisation of the TDP-43 inclusions accumulating in the cortex or spinal cord of patients or formed *in vitro* indicate contradicting reports (1–4).

Objectives: The aim of the present study is to clarify the structural and morphological nature of the TDP-43 aggregates and assess their toxic activity.

Methods: We have over-expressed TDP-43 in bacterial *Escherichia. coli* cells and found that the expressed protein spontaneously aggregates as inclusions bodies (IBs). Since bacterial IBs contain both native-like proteins and amyloid fibrils, depending on the intrinsic propensity of the expressed protein, IBs represent a suitable system to investigate the type of protein aggregates formed spontaneously by TDP-43. We have thus purified IBs containing TDP-43, and control IBs devoid of TDP-43 and analysed them with a number of biophysical techniques.

Results: We found that the TDP-43 component of IBs does not increase thioflavin T fluorescence or cause a red-shift of the Congo Red optical absorption. The TDP-43 component of IBs was found to possess a random-coil secondary structure, as detected with far-UV circular dichroism and infrared spectroscopy. Proteolytic digestion with proteinase K shows that TDP-43 aggregates are digested rapidly, revealing a non-compact, non-amyloid structure. Finally, the analysis of the IBs with atomic force microscopy shows that TDP-43-containing IBs have a more irregular structure than control IBs.

We have also tested the toxicity of TDP-43 and control IBs to neuroblastoma SH-SY5Y cells, monitoring the decrease in MTT reduction, the accumulation of intracellular ROS and the increase of caspase-3 activity as indicators of cellular toxicity. The results indicate that TDP-43 IBs, unlike the control IBs, are toxic to SH-SY5Y cells when they are both added to the extracellular medium and brought inside the cells using a transfection method based on a cationic amphiphilic molecule.

Discussion: Overall, these results show that TDP-43 aggregates accumulating into IBs appear to be structurally disordered and morphologically amorphous, thus showing a non-amyloid nature. In this form, the aggregates appear toxic highlighting that protein aggregate toxicity is coupled to non-amyloid aggregation for TDP-43.

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P254   MOTONEURON AND MUSCLE-SELECTIVE REMOVAL OF ALS-RELATED MISFOLDED PROTEINS

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Keywords: protein misfolding, muscle, autophagy

Background: Amyotrophic lateral sclerosis (ALS) occurs in clinically indistinguishable sporadic (sALS) or familial (fALS) forms. Most of the fALS-related mutant proteins identified so far, such as mutant SOD1, TDP-43, FUS, are prone to misfold; also the product of the mutant C9ORF72 gene aberrantly codes for small highly hydrophobic dipeptides. Both misfolded proteins and hydrophobic peptides accumulate into insoluble proteinaceous material inside motor neurons. This material must be cleared away from cells with the assistance of the molecular chaperones. Chaperones may act on aberrant proteins either by assisting their refolding or by directing them to degradation through the proteasome (UPS) or the autophagic system.

Results: Motor neurons are very sensitive to misfolded protein toxicity, but other cell types, such as astrocytes, oligodendrocytes, and muscle cells could also be affected by their presence. Notably, muscle-restricted expression of mutant SOD1 (mutSOD1), responsible for some fALS, induces muscle atrophy and motor neuron death. We found that several genes are altered in the skeletal muscle of mutSOD1 mice. In fact, we observed upregulation of specific muscle genes, such as MyoD, myogenin, and also of several components of cell response to proteotoxicity (atrogin-1,

HspB8, Bag1, and Bag3). Similar changes were found to occur in cultured ALS myoblasts.

We then compared the potential mutSOD1 toxicity in motor neuron (NSC34) and muscle (C2C12) cells. Initially, we found that muscle ALS models possess much higher chymotryptic proteasome activity and autophagy power than motor neuron ALS models. The mutSOD1 molecular behaviour was also very different. MutSOD1 clearance was much higher in muscle than in motor neurons, and the misfolded protein formed aggregates and impaired proteasome only in motor neurons. The motor neuronal cells were also more sensitive to superoxide-induced oxidative stress. In muscle cells, mutSOD1 remained soluble even after proteasome inhibition, possibly because of high mutSOD1 autophagic clearance. Finally, N-terminal TDP-43 fragment accumulated in NSC34, but not in C2C12 cells. In the case of TDP-43, proteasome inhibition resulted in a large accumulation of both wt and N-terminal fragment of TDP-43.

Discussion: Therefore, our results suggest that muscle cells differentially manage misfolded mutSOD1 and TDP-43 and their toxicity in muscle may not directly depend on aggregation.

P255   ACTIVATION OF TRANSFORMING GROWTH FACTOR-BETA/SMAD SIGNALING REDUCES AGGREGATE FORMATION OF MISLOCALIZED TAR DNA-BINDING PRTOTEIN-43

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Keywords: transforming growth factor-beta, phosphorylated Smad2, TDP-43

Background: We have previously reported that phosphorylated Smad2/3 (pSmad2/3), the major intracellular mediators of transforming growth factor (TGF)-beta signaling, abnormally accumulated in TDP-43 inclusions of anterior horn cells (AHCs) in amyotrophic lateral sclerosis (ALS). The nuclear pSmad2/3 signal is decreased in AHCs bearing TDP-43 inclusions in comparison with those without these inclusions. It suggested that neuroprotective effect of TGF-beta might be disturbed in ALS. TGF-beta is multifunctional cytokine that regulates cell growth, differentiation, and apoptosis. Furthermore, TGF-beta activates autophagy. In cultured cells, the nuclear localization signal (NLS) deletion mutant of TDP-43 (ΔNLS-TDP-43) has been reported to aggregate in the cytoplasm in the presence of a proteasome inhibitor.

Objective: To investigate the possible pathophysiological linkage between TDP-43-positive inclusions and TGF-beta/Smad signaling system.

Methods: We constructed a plasmid of the nuclear localization signal deletion mutant of TDP-43, and transfected it into HEK293T cells. TDP-43-positive inclusions were formed in the cytoplasm of the HEK293T cell under proteasome inhibi-

tion. Plasmids of Smad2 protein and constitutive active or dominant negative forms of TGF-type1 receptor were also transfected into the HEK293T cells. The direct effects of TGF-beta/Smad signaling on the aggregation formation were analyzed using immunocytochemistry and Western blotting.

Results: The aggregates formed in the cytoplasm of the HEK293T cells contained ubiquitinated, phosphorylated, and fragmented TDP-43, consistent with the essential features of the human pathology. Moreover, the aggregates were co-localized with phosphorylated Smad2 under continuous TGF-beta stimulation. These aggregates were immunopositive for p62 and LC3. Overexpression of Smad2 reduced the amount of cytoplasmic aggregates in HEK293T cells. TGF-beta stimulation augmented this reduction effect in a dose-dependent manner.

Discussion: As phosphorylated Smad2 was sequestered within cytoplasmic aggregates, TGF-beta/Smad signaling might be disturbed in the aggregation cells. The co-localization of TDP43 aggregates with p62, and LC3 suggests that autophagy may take part in the degradation of the aggregates. Stimulation with TGF-beta/Smad signaling may result in overcoming this sequestration of pSmad2 by TDP-43 aggregates, and autophagy could be induced under proteasome inhibition.

Conclusions: Our data indicate that activation of TGF-beta/Smad signaling system is protective against aggregate formation of cytoplasmically mislocalized TDP-43, and may be a potential therapeutic approach to delay the progression of ALS.

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P256   FUNCTIONAL EFFECTS OF TDP-43 MUTATIONS IN HUMAN IPSC-DERIVED MOTOR NEURONS AND GENOMIC DNA MODELS

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Keywords: TDP-43, calcium signaling, iPSC derived motor neurons

Background: The transactive-response DNA-binding protein (TDP-43) is a major protein component of the characteristic ALS neuronal cytoplasmic inclusions. Mutations in the gene encoding TDP-43 have been identified in

sporadic and familial ALS cases, but molecular mechanisms of disease associated with TDP-43 pathogenic mutations remain to be elucidated.

Objectives: The aim of our work is to investigate pathological and physiological phenotypes associated with TDP-43 pathogenic mutations in human motor neurons, using improved genomic DNA expression models and induced pluripotent stem cell (iPSC)-derived motor neurons.

Methods: To study the molecular mechanisms of TDP-43 mutations, we generated novel *in vitro* cellular models by site-specific bacterial artificial chromosome (BAC) integration in HEK293 of a fluorescently tagged full-length human genomic *TARDBP* locus carrying one of two ALS-associated mutations (A382T or M337V). Induced pluripotent stem cells were generated from healthy volunteers and differentiated to mature motor neurons, which were characterized by immunostaining, electrophysiology and calcium imaging. TDP-43 BACs were transduced into iPSC-derived motor neurons by Herpes simplex virus type 1 (HSV-1), and functional investigations were carried out.

Results: While both exogenous and endogenous TDP-43 were mainly localized to the nucleus in HEK293 cells, a higher frequency of cytoplasmic TDP-M337V was observed compared to wild-type TDP-Ypet. Mis-localization was exacerbated by the presence of oxidative stress. In iPSC-derived motor neurons transduced with TDP-43 BACs, cytoplasmic translocation was observed at high levels of oxidative stress, without significant differences between TDP-43 mutant and wild-type TDP-Ypet. Increased susceptibility to cell death was detected in HEK293 cells carrying M337V mutations, with 38% of cells positive for cleaved caspase 3, compared to only 18% of cells carrying wild-type TDP-Ypet, while no significant differences in survival assays were observed in iPSC-derived motor neurons carrying TDP-43 mutations. Calcium signaling from the endoplasmic reticulum (ER) was found to be impaired in HEK293 clonal cell lines carrying TDP-43 mutations, which showed 50% reduction in ER Ca^{2+} concentration and decreased Ca^{2+} amplitude compared to TDP-Ypet. Calcium dysregulation correlated with high levels of Bcl-2 in cells carrying M337V and A382T mutations. Knockdown of Bcl-2 restored amplitude of ER Ca^{2+} release in TDP-M337V similar to the levels of ER Ca^{2+} release amplitude detected in TDP-Ypet.

Discussion and conclusion: Using a novel full-length genomic TDP-43 BAC construct tagged with the fluorescent protein Ypet, our study shows that two TDP-43 ALS-specific mutations, A382T and M337V, increase the probability of cytoplasmic mis-localization in HEK293 cell lines and correlate with ER Ca^{2+} dysregulation, as well as with cell death. High levels of Bcl-2 were associated with TDP-43 mutations, and its knockdown restored ER Ca^{2+} dynamics, suggesting a novel potential mechanistic link between TDP-43 and Bcl-2 in the regulation of ER Ca^{2+} release.

P257   TDP-43 PROTEINOPATHY: LOSS- AND GAIN-OF-FUNCTION DISEASE CELL MODELS

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Background: TDP-43 protein forms pathological inclusions in affected tissues of ALS and FTLD patients. In disease conditions, TDP-43 mislocalizes from the nucleus to the cytoplasm and it is not well-established yet if TDP-43-associated toxicity is due to a nuclear loss- or a cytoplasmic gain-of-function mechanism. The loss-of-function hypothesis is based on the observed clearance of TDP-43 from the nucleus together with its sequestration into aggregates that may affect its main pre-mRNA splicing activity and its cytoplasmic activities, such as mRNA transport, stability and translation. In the gain-of-function hypothesis, the presence of cytoplasmic TDP-43 aggregates is supposed to trigger cell death by affecting important and still unknown cellular processes.

Objectives: To better investigate this issue, we studied how and whether the expression and protein content of one of TDP-43 mRNA targets, the neurotrophic factor Progranulin (*PGRN*), is altered in TDP-43 proteinopathy cell models.

Methods: The loss-of-function cell models were obtained by knocking down TDP-43 in human neuroblastoma SKNKBE and murine motoneuron-like NSC34 cells, while the gain-of-function model by over-expressing the TDP-43 C-terminal Gln/Asn-rich region (12X-Gln/Asn), recently shown to induce aggregation.

Results: Since we previously reported that TDP-43 post-transcriptionally regulates *Pgrn* mRNA stability, influencing also its protein level in NSC34 cells, we confirmed that TDP-43 depletion determined a significant increase in PGRN protein levels also in the human SKNBE cells. The increased PGRN content was observed also for the secreted protein in the medium, but specifically in the mouse NSC34 cells and not in SKNKBE cells. Conversely, the presence of TDP-43-positive aggregates in the gain-of-function cell models did not seem to change the content of both the endogenous and the secreted PGRN protein in the human and mouse cell lines.

Discussion and conclusion: PGRN levels need to be maintained within certain physiological ranges because a reduced content is pathogenetic in FTLD patients carrying non-sense mutations in *Pgrn* gene and an increased level is observed in ALS-affected tissues. Our data show that TDP-43 depletion rather than its sequestration into aggregates is able to influence the protein amount of its target *Pgrn*, suggesting that reduced levels of TDP-43 are likely to alter the neuronal and/or the neighbouring cell metabolism. Our disease cell models provide a useful tool to better investigate the molecular mechanisms and the cellular pathways involved in TDP-43 proteinopathy *in vitro*. However, the differences observed in the post-transcriptional regulation of *Pgrn* in human and in the mouse cell lines should be carefully taken into account when studying TDP-43 and its dysfunction in different disease models.

P258   ALTERNATIVE SPLICING OR POLYADENYLATION, WHICH IS THE MAJOR MECHANISM FOR AUTO-REGULATION OF TDP-43?

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Keywords: TDP-43, nonsense-mediated mRNA decay, polyadenylation,

Background: TAR DNA-binding protein-43 (TDP-43), a predominantly nuclear protein, plays a key role in the pathogenesis of amyotrophic lateral sclerosis. Accumulating evidence suggests that TDP-43 levels in the nucleus should be strictly regulated especially in central nervous system. TDP-43 is auto-regulated via binding to its own 3'UTR. Mechanisms following auto-regulation remain unclear.

Objective: To investigate the mechanism of TDP-43 auto-regulation.

Materials and methods: Flp-In 293 cell lines stably expressing myc-tagged wild-type TDP-43 cDNA was used to investigate elevated TDP-43 effects on endogenous TDP-43 mRNA. Minigene-containing wild-type exon-6 of TDP-43, which includes TDP-43-binding region, was used to investigate depleted TDP-43 effects on the transcripts. In situ hybridization, northern blotting of polyA (+) RNA in cytoplasmic and nuclear extraction, and 3'-end qRT-PCR were performed to evaluate distribution and expression levels of each isoform of the transcripts.

Results: Northern blot analysis and qRT-PCR revealed that TDP-43 mRNA was alternatively polyadenylated. The levels of transcripts using distal polyadenylation sites were increased with increasing TDP-43 levels. In situ hybridization and northern blot analysis of RNA extracted from nucleus or cytoplasm showed that substantial amounts of TDP-43 mRNA with distal polyadenylation were located in the nucleus. The increasing level of TDP-43 reduced its own mRNA from cytoplasm, whereas the TDP-43 mRNAs in the nucleus with distal polyadenylation sites were unchanged. The mRNA with different polyadenylation sites, however, did not alter its stability. Northern blot analysis, upon cycloheximide treatment showed shorter isoforms, excited 2 or 3 introns in exon-6. These isoforms fulfilled the criteria for nonsense-mediated mRNA decay (NMD).

Discussion and conclusion: Increasing TDP-43 undergoes excision intra-exonic introns, resulting in the reduction of its own mRNA via NMD pathway. In addition, the amounts of TDP-43 altered the polyadenylation sites in combination with alternative splicing, which regulates intracellular distribution (nuclear or cytoplasm) of the mRNA. These mechanisms are collaboratively involved in the auto-regulation of TDP-43.

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P259   DISTINCT SPLICING PATTERNS FOR TDP-43 AND FUS RNA-BINDING PROTEINS IN NEURAL-LIKE CELLS

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Keywords: splicing, TDP-43, FUS

Background: The two RNA-binding proteins TDP-43 and FUS are implicated in the pathogenesis of ALS and FTD, and they both share similar features and functional activities in post-transcriptional regulation of gene expression. Although they similarly regulate pre-mRNA splicing in the nuclear compartment and mRNA transport into neurites, to what extent their function is overlapping biologically is not established yet. As TDP-43 and FUS lose their main nuclear localization in ALS-affected tissues, RNA metabolism and alternative splicing mediated by these proteins may be defective in disease conditions and trigger neurodegeneration.

Objectives: Our aim was to define the effects of the nuclear depletion of TDP-43 and FUS proteins on the alternative splicing patterns and gene expression profiles in neural-like cells.

Methods: TDP-43 and FUS were knocked down in human neuroblastoma SKNBE cells and alternative splicing, and gene expression analyses were performed using the Affymetrix Human Exon 1.0ST GeneChips. EASANA analysis tool was used for data visualization (GenoSplice). Q-PCR and RT-PCR were employed to validate gene expression and splicing changes, respectively.

Results: We found 265 and 64 genes differentially spliced (splicing index, ≥ 2) in TDP-43 and FUS knocked-down SKNBE cells, respectively, 25 of which were commonly misspliced. Moreover, silencing of TDP-43 and FUS resulted in 386 and 143 genes differentially expressed, respectively, with 56 common genes. In particular, almost 60% of the differentially expressed genes for both TDP-43 and FUS were down-regulated.

Functional annotation analyses revealed that genes alternatively spliced in condition of TDP-43 and FUS depletion belong to different GO categories enriched in GTPase regulator activity and apoptosis for TDP-43 and in neuron differentiation for FUS. On the contrary, differentially expressed genes in TDP-43- and FUS-silenced cells showed overlapping GO categories, including apoptosis, cell migration and response to oxygen levels.

Importantly, our microarray data for TDP-43 have confirmed some of the alternatively spliced transcripts recently identified by other groups, including *TNIK* and *POLIDIP3* genes. However, to further confirm our experimental results, we validated by RT-PCR some alternatively spliced transcripts selected on the basis of their reported involvement in neuronal cell metabolism. In parallel, changes in the expression of selected genes (fold change > 2) were also confirmed by quantitative real-time PCR.

Discussion and conclusions: Our findings indicate that TDP-43 regulates the alternative splicing and expression pattern of specific targets compared to FUS, although common targets were identified, too. The recent literature data also seem to support the idea that TDP-43 and FUS proteins behave differently in regulating alternative splicing and are not associated in the same ribonucleoprotein complexes. The altered transcriptomes emerging from our study will help give further insights into the potential cellular pathways disrupted by the loss of TDP-43 and FUS activity in neurodegenerative diseases.

P260　ALS-ASSOCIATED FUS MUTANTS RETAIN SPLICEOSOMAL SNRNPS IN THE CYTOPLASM

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Keywords: FUS, SMN, alternative splicing

Background: Motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are associated with defects in proteins involved in RNA metabolism (TDP43 and FUS, and SMN, respectively). SMN, the causative factor in SMA, is crucial for the biogenesis of the spliceosomal snRNPs. FUS forms cytoplasmic aggregates, as a consequence of disturbed nuclear import due to disease-causing mutations. It is extremely likely that the cytoplasmic aggregates are cytotoxic because they trap important factors; the nature of these factors, however, remains to be elucidated.

Objectives: We investigated whether mutant FUS and reduced SMN might disturb the same pathway, namely the biogenesis of snRNPs, looking for mutual interactions and disturbed expression of these factors.

Methods: Mouse motoneuronal NSC34 cells transfected with wild-type or mutant FUS were used as cellular models of ALS. qPCR coupled to immunoprecipitation, as well as to immunofluorescence analysis and fluorescence *in situ* hybridisation (FISH), was used to assess expression, FUS binding, and subcellular distribution of the snRNPs. A splice reporter plasmid including the exon 7 of human SMN2 was used to monitor alternative splicing variations by FUS.

Results: To test the hypothesis that FUS might be involved in snRNP biosynthesis, we checked for a physical association with SMN, the catalyst of snRNP assembly, and with the snRNAs themselves. We found that FUS and SMN associated with each other, and FUS bound to Sm-snRNPs. Mutations in FUS did not affect association with the snRNPs, but caused their retention in the cytoplasm. Since the total snRNP concentration did not change, this reduced the availability of functional snRNPs in the nucleus. As a result, alterations in the alternative splicing of a reporter plasmid were observed.

Discussion and conclusion: Our results suggest that aggregated FUS may indeed be toxic because they sequester spliceosomal snRNPs in the cytoplasm, lowering their availability in the nucleus and thus leading to changes in alternative splicing patterns. In this sense, the FUS mutations and genetic depletion of SMN interfere with the same pathway, which might represent a unifying theme in the FUS-related ALS and SMA.

P261　THE ALS-ASSOCIATED PROTEIN FUS/TLS IS A COMPONENT OF THE CELLULAR RESPONSE TO DNA DAMAGE

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Keywords: FUS/TLS, DNA damage response, RNA processing

Background: Fused in sarcoma/translocated in sarcoma (FUS/TLS) is a member of the hnRNP family of RNA-processing factors that bind thousands of pre-mRNAs and can regulate their splicing, raising the possibility that ALS associated with FUS mutation may involve loss-of-function defects in RNA processing. However, how defects in RNA processing might lead to neurodegeneration is unclear. One possibility is that FUS might be important to maintain the expression of genes that have acquired DNA damage, to prevent inappropriate mRNA splicing and/or polyadenylation. DNA suffers from an intrinsic level of instability and from attack by reactive oxygen species and, as a result, accrues DNA lesions that can block transcription. Since the cumulative number of DNA lesions encountered by a gene is dependent on cell age, DNA damage poses the greatest threat to gene transcription in long-lived cells such as neurons.

Objectives: To examine whether FUS is a component of the response machinery to DNA damage in mammalian cells.

Methods: KU58948, or KU55933, was used to inhibit PARP1 or ATM, respectively. GFP-positive cells were irradiated with a 351-nm UVA laser. UVA (0.44 J/m^2) was introduced to an area of ~12 μm x 0.1 μm, and images were captured at 15-s intervals. Pre-sensitisation was carried out as above, and individual cells were irradiated with 4.4 J/m^2 UVA.

Results: We observed that both mouse and human GFP-FUS were rapidly recruited to sites of UVA-induced oxidative DNA damage, which include both single- and double-strand breaks. Importantly, endogenous FUS similarly accumulated at these sites as measured by two separate anti-FUS antibodies, ruling out that this response was an effect of overexpressing GFP-tagged protein. Moreover, the recruitment of FUS to the DNA lesions was dependent on the activation of poly (ADP-ribose)-polymerase-1 (PARP-1), a critical protein sensor of chromosomal DNA strand breaks. Intriguingly, the ALS-associated R521G mutation in the C-terminal domain of FUS significantly reduced FUS accumulation at sites of UVA-induced DNA damage in both A549 and HeLa cells. In addition, treatment of mammalian cell lines A549 and HeLa Cells expressing GFP-FUS with camptothecin (CPT; a DNA single-strand-break inducing toxin) led to rapid accumulation of GFP-FUS in the nucleoli. We observed similar results for endogenous FUS in U20S cells. Interestingly, this accumulation was reversible following CPT removal, consistent with the rapid repair of Top1-induced single strand breaks.

Conclusions: These data suggest that FUS relocalises in response to DNA damage, including damage that induces transcriptional stress by blocking the progression of RNA polymerases. Our data highlight a new aspect of FUS function, and supports a model in which FUS is an important component of the mechanism/s by which cells respond to transcriptional stress.

P262   AUTOSOMAL DOMINANT INHERITANCE OF RAPIDLY PROGRESSIVE JUVENILE-TYPE ALS DUE TO A FUS MUTATION

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Keywords: FUS, familial, truncation

Background: Approximately 5% of ALS cases are familial. Mutations in the gene encoding FUS/TLS (fused in sarcoma/translocated in liposarcoma) are responsible for about 4% of familial and < 1% of sporadic disease, and a high frequency of cases have a young onset and an aggressive clinical course. The FUS protein contains a nuclear localisation signal (NLS) at the extreme C terminal, a common site for mutations. *In vitro* studies suggest that mutations can cause FUS mislocalisation from the nucleus to the cytoplasm, and subsequent aggregation in stress granules.

Objectives: Identification of a disease-causing mutation in a family with an unusually severe form of autosomal dominant ALS, and initial functional studies using cell culture.

Methods: We undertook whole exome sequencing in a family with an unusually severe ALS phenotype with onset in adolescence or early adult life. We then characterised the cellular effects of an identified FUS mutation using site-directed mutagenesis to create an expression construct which was expressed in cell lines and primary neurons. The effect of this mutation on FUS distribution was compared to that on wild-type and P525L mutant FUS.

Results: DNA from members of a family with an aggressive form of lower motor neuron predominant ALS with onset from 15 to 30 years was analysed using whole exome sequencing, demonstrating a heterozygous AG deletion in exon 14 of FUS (c.1509_1510delAG). This frameshift results in a truncated protein lacking the nuclear localisation signal. The mutation was expressed in neuronal cell lines and primary neurons. The effect of this mutation on FUS distribution and stress granule formation, compared to overexpressing wild-type and P525L mutant FUS, demonstrated a severe cellular phenotype, in keeping with previously described truncation mutations.

Discussion: This is the first reported family with several members bearing a severe truncating mutation in FUS. Truncation mutations tolerated long enough to allow vertical transmission have not previously been described, presumably due to the severity of the cellular phenotype. These studies will further examine the hypothesis that mutations causing clinically aggressive disease are paralleled by greater *in vitro* changes. This supports the argument that failure of nuclear localisation is central to the toxicity of mutant FUS.

Conclusions: Severe truncating mutations can rarely be transmitted prior to disease onset. Further work will examine the effect of this mutation on cellular homeostasis in primary motor neurons.

P263   EVALUATION OF THE ROLE OF SMN (SURVIVAL OF MOTOR NEURON) PROTEIN IN PATHOLOGICAL FUS STRESS GRANULES IN PRIMARY NEURONS

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Keywords: FUS, SMN, stress

Background: Mutations in the gene encoding the RNA-binding protein FUS (Fused in sarcoma) cause a subtype of ALS characterised pathologically by cytoplasmic insoluble FUS inclusions in neurons and glia. Stress granules are storage and sorting sites for stalled translation complexes, and incorporation of mutant FUS into cytoplasmic stress granules has been proposed to be a precursor of insoluble FUS inclusions. Despite the important advances in our knowledge of FUS pathological deposition, the mechanism of toxicity of mutant FUS is less clear. Recent studies have suggested that wild-type FUS interacts with nuclear SMN, the spliceosome

and is involved in nuclear Gem formation, all of which are perturbed by pathogenic FUS mutations. Evidence of SMN incorporation into cytoplasmic stress granules, and pathological evidence of SMN immunoreactivity in FUS-positive inclusions at autopsy, supports the need for a further evaluation of SMN in FUS-mediated toxicity.

Objectives: To examine the involvement of SMN in pathological FUS stress granules in primary cortical and motor neurons.

Methods: Primary motor neuron and cortical cultures were established from embryonic E13.5 and E16.5 mice, and were transfected by magnetotransfection with pcDNA-based constructs expressing wild-type, R521C or P525L FUS. NSC-34 cells were transfected with the same constructs using Lipofectamine. Neurons and NSC-34 cells were stressed by treatment with 0.5mM sodium arsenite, fixed and stained for FUS, SMN and the stress granule marker PABP-1. Stringent analyses were performed to clarify SMN:FUS co-localisation in cytoplasmic stress granules.

Results: We found R512C and P525L FUS incorporated readily into PABP-1-positive stress granules in both cortical and motor neurons, consistent with previous reports. Here, we provide further clarification of the extent of SMN incorporation into FUS-containing stress granules in both motor and cortical neurons.

Discussion and conclusion: Previous reports, principally using neuronal or non-neuronal transformed cell lines, have shown the ability of SMN to form cytoplasmic stress granules when exposed to cellular stress. We have used two different populations of primary neurons and a motor neuron-like cell line to study further the role of SMN in native and mutant FUS-containing stress granules. Although further studies are required, the current findings suggest that results from transformed cell lines may not be applicable to primary neurons and further clarify the role of SMN in cytoplasmic stress granules.

P264   UBIQUILIN 2 MUTATIONS INDUCE ENDOPLASMIC RETICULUM STRESS

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Keywords: Calreticulin, CHOP, ER stress, UBQLN2, XBP-1

Background: Mutations in *ubiquilin 2*, an ubiquitin-like protein which regulates protein degradation, have been shown to cause dominant X-linked ALS and ALS/dementia. Previous studies have shown that endoplasmic reticulum (ER) stress is a common pathogenic mechanism shared in both sporadic and familial ALS cases. Ubiquilin1 gene (*UBQLN1*) variants have been shown to be associated with an increased risk of Alzheimer's disease, and additionally ubiquilin1 has been demonstrated to interact with

ER-associated proteins, presenilin 1 and presenilin 2, as well as with the molecular chaperone protein disulphide isomerase (PDI). Furthermore, ubiquilin1 has been shown to associate with the ER during hypoxia-induced ER-stress, and works co-operatively with PDI to attenuate C/EBP homology protein (CHOP) induction.

Objectives: To investigate the relationship between the ER and ubiquilin 2.

Methods: Neuro2A and COS-7 cells were transiently transfected with wild-type (WT) and mutant ubiquilin 2 constructs (P497H and P506T) and fixed with 4% PFA or 100% methanol at 48 h. Immunocytochemistry using antibodies against CHOP and X-box binding protein 1 (XBP-1) were used to detect ER stress, and ER marker calreticulin was used to observe changes in ER morphology. Neuro2A cells were also transfected with ubiquilin 2 constructs and harvested at 24 h post-transfection. Immunoblotting was performed with antibodies against CHOP, XBP-1, IRE1, Bip and P-PERK.

Results: Immunocytochemistry using Neuro2A and COS-7 cells transfected with ubiquilin 2 and stained with calreticulin revealed no perturbations in ER morphology. Ubiquilin 2 ALS mutants (P497H and P506T) demonstrated a significant increase in nuclear CHOP immunoreactivity, demonstrating activation of ER stress 48 h post-transfection. Western blot analysis at 24 h post-transfection demonstrated an upregulation of CHOP and Bip for ubiquilin 2 mutants (P497H, P506T).

Discussion and conclusion: The results obtained in this study demonstrate that ubiquilin 2 mutations induce ER stress, adding ubiquilin 2 to a growing list of proteins linked to ALS that demonstrate ER dysfunction.

P265   YEAST MODEL EXPRESSING ALS-LINKED P56S-VAPB EXHIBITS INCREASED SENSITIVITY TO OXIDATIVE AND ENDOPLASMIC RETICULUM-INDUCED STRESSES

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Keywords: VAPB, oxidative stress, endoplasmic reticulum stress

Background: Some mutations in the gene encoding VAPB are responsible for familial amyotrophic lateral sclerosis type 8 (FALS8). Despite its function not being fully established in mammals, its homologous protein in *Saccharomyces cerevisiae*, SCS2, is involved in different aspects of cell biology of the endoplasmic reticulum, lipid metabolism and in unfolded protein response (UPR) which can result in apoptosis.

Objectives: Since mutations in the VAPB gene result in ALS and the same mutation may show very different clinical phenotypes, we seek to study the susceptibility to oxidative stress and endoplasmic reticulum stress as factors underlying this clinical heterogeneity. Therefore, we performed an integrated analysis in order to understand the molecular

mechanisms involved in this disease using *S. cerevisiae* as a model.

Results: BY4741 strain was transformed with expression vectors containing genes of wild or mutant (P56S) human VAPB and a control strain with an empty expression vector. Growth curves and serial dilutions were made to assess the viability and sensitivity of *S. cerevisiae* under treatment with different concentrations of hydrogen peroxide (an oxidative stress inducer) and DTT (an endoplasmic reticulum stress inducer). As a parameter of redox state of the cells, the ratio of reduction to oxidized glutathione by HPLC with electro-chemical detection was determined by enzymatic assays in normal conditions and under treatment with hydrogen peroxide and DTT.

Discussion and conclusion: Our results show lower growth and viability of cells carrying the mutant gene (VAPB[P56S]) when compared to those carrying the wild-type gene and the control strain both under treatment with stress inducers and under normal conditions. Interestingly the expression of wild-type VAPB was also toxic suggesting that high levels of VAPB can modify some metabolic pathways in yeast cells. The ratio of reduced to oxidized glutathione in normal conditions was two-fold in the control strain than in the VAPB[P56S] mutant strain. Under conditions of induced oxidative stress, VAPB[P56S] mutants also displayed a lower ratio of reduction to oxidized glutathione and, comparatively to those data obtained in normal conditions, this ratio was even smaller. Similar results were obtained in the treatment with DTT, suggesting a link between oxidative and endoplasmic reticulum stresses.

P266   THE ROLE OF RBM45 IN ANTIOXIDANT RESPONSES IN ALS

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Keywords: RNA binding proteins, oxidative stress, NRF2

Background: Numerous signaling pathways have been proposed to contribute to ALS disease pathogenesis, including oxidative stress/injury (1). RNA-binding proteins FUS and TDP-43 have been implicated in the disease etiology, although their exact role in pathogenic mechanisms remains unclear. Our group has recently described the involvement of a new RNA-binding protein, RBM45 in ALS (2). Increased levels of RBM45 were detected in the cerebrospinal fluid of ALS patients, and the protein was localized to cytoplasmic inclusions that often co-localized with TDP43- and ubiquitin-positive aggregates.

Objectives: In this study, we further characterize RBM45 function and subcellular distribution within cultured cells and primary neurons using various molecular and biochemical approaches. We generate various tools to overexpress or knock-down full-length RBM45 as well as various truncations mutants to examine their effect on cellular function and viability.

Results: We found RBM45 to bind and stabilize KEAP, the inhibitor of the antioxidant response transcription factor NRF2. Overexpression of RBM45 increases KEAP levels, inhibiting NRF2 and the antioxidative response element signaling pathway, thus increasing cellular death in response

to oxidant insult. We further mapped the functional region of the protein responsible for such effects.

Discussion and conclusion: Our findings define a novel role of RBM45 in the regulation of the oxidative status of the cell. Specifically, we show a detrimental effect of RBM45 on cellular response to oxidative injury. Given that oxidative damage plays a major role in neuronal death, these results provide the first link between an RNA-binding protein that can form cytoplasmic inclusions and the KEAP/NRF2/antioxidant response element signaling pathway in ALS.

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P267   EVALUATION OF EXPRESSION AND LOCALIZATION OF ELAV PROTEINS IN ALS

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Keywords: RBPs, neurodegeneration

Background: Altered RNA metabolism contributes to the pathogenesis of neurodegeneration and several RNA-binding proteins (RBPs) have been implicated in ALS pathogenesis (1). Indeed post-transcriptional regulatory mechanisms mediated by RBPs are fundamental for the development of the nervous system and its maintenance. ELAV proteins are RBPs which exert a pivotal role in modulating several aspects of RNA metabolism.

HuB, HuC and HuD represent the neuron-specific members of ELAV family (nELAVproteins), while HuR is ubiquitously expressed. In accordance with their function, nELAVs are mainly cytoplasmic, while HuR is characterized by a nucleus-cytoplasm shuttling ability. ELAV genes are spatially and temporally regulated, showing different expression patterns within the developing and adult nervous system.

Objectives: In this study, we aimed to explore potential variations in ELAVs expression in an *in vitro* cellular model of neurodegeneration and in samples from sporadic ALS (SALS) patients and controls. We focused on HuR and HuD since their involvement in neurodegenerative processes has been largely reported (2).

Methods: Human neuroblastoma SH-SY5Y cells treated with 1mM H_2O_2 for 30 and 60 min have been used. ELAV protein expression and subcellular localization were evaluated using immunocytofluorescence and Western botting (WB), while mRNA levels were quantified by real-time PCR. HuR phosphorylation in peripheral blood mononuclear cells (PBMCs) from SALS patients and healthy controls were

assessed using immunoprecipitation coupled with WB. Immunohistochemistry and real-time PCR experiments were carried out in cerebral motor cortex tissues from SALS patients and controls to evaluate HuR and HuD protein expression/localization and mRNA levels, respectively.

Results: In our *in vitro* cellular model, we did not observe significant variations in HuD intracellular distribution, while we detected an increased HuR expression in the cytoplasm under oxidative stress condition. No significant variations were reported in HuD and HuR mRNA levels after the treatment. In PBMCs from SALS patients compared to controls, we could not appreciate changes in HuR expression and distribution; nevertheless, we found a specific increase in HuR phosphorylation, suggesting that this protein is more activated in the pathology. Finally, in line with the findings reported in our cellular model, we observed that, although there were no significant variations in mRNA levels, HuR and HuD protein expression was increased in the cytoplasm and in the perinuclear area in cerebral motor cortex from SALS patients compared to healthy individuals.

Discussion and conclusion: These preliminary data suggest a possible involvement of ELAVs protein in neurodegeneration. Further studies will be needed to confirm our data on SALS patients and to identify the functional role of ELAVs protein in ALS.

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P268   CAUSES AND CONSEQUENCES OF MICRORNA MALFUNCTION IN ALS

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Keywords: RNA, microRNA, dicer

Background: ALS-causing mutations, recently discovered in genes encoding for RNA-binding proteins, encourage exploration of RNA-related processes in ALS pathogenesis. microRNAs provide homology-based silencing that is essential for brain integrity.

Objective: We tested the causes and consequences of microRNA dysregulation in ALS.

Methods and results: We are employing molecular biology, mouse genetics and human molecular histopathology. Our study reveals global downregulation of microRNAs in ALS lower-motoneurons from the lumbar region of sporadic and familial cases, but not in RNA extracted from surrounding, neuron-depleted ventral horn tissue or from the neurons of Clarke's column in the same autopsies.

We sought molecular mechanistic insight for dysregulation of microRNAs in tissue culture studies. We show that the canonical microRNA bioprocessing pathway is disrupted at

the level of Dicer1 activity, by ALS-causing mutants FUS495X, FUSR521G, TDP-43A315T and TDP-43M337V. Dicer impairments result in pre-microRNA precursor accumulation and mature microRNA downregulation (1). Accordingly, loss of Dicer1 and microRNAs activity in a Dicer1 conditional knockout in spinal motoneurons resulted in degeneration of spinal motoneurons and in denervation-dependent muscle atrophy (2). Our newest pieces of data provide mechanisms for Dicer dysregulation and suggest that cellular stress impact the activity of the Dicer complex. We will present unpublished data that dissect how stress signalling impairs Dicer activity in ALS.

Conclusions: microRNA plays a key role in brain integrity and is probably involved in several forms of ALS. Dysregulation of Dicer is related to stress signalling and suggests that potentiating of Dicer activity may be beneficial in ALS. Our data link post-transcriptional regulation to stress signalling in ALS.

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P269   *IN VITRO* CHARACTERIZATION OF RBM45, A NEW RNA-BINDING PROTEIN IMPLICATED IN ALS AND FTLD

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Keywords: RNA-binding protein, inclusion, CLIP-seq

Background: A major advancement to our understanding of the ALS and FTLD pathogenesis was the identification of mutations in the RNA-binding proteins TDP-43 and FUS in familial cases of ALS and FTLD, and the observation of cytoplasmic aggregates of these proteins in familial and sporadic ALS and FTLD. However, the motor neuron degeneration mechanism remains unknown. Our laboratory has recently identified a new RNA-binding protein, RBM45, with pathologic alterations in ALS and FTLD. RBM45 containing cytoplasmic inclusions were observed in both ALS and FTLD patients (1). RBM45 also colocalized with TDP-43 and ubiquitin inclusions in affected neurons. However, very little is known about the physiological functions and RNA-binding targets of RBM45.

Objective: We have used *in vitro* cultured cells to characterize the structural functions of RBM45. We have also identified the RNA-binding targets of RBM45 to study the RNA-mediated pathways of neurodegeneration.

Methods: We have used *in vitro* culture cells (HEK293, Neuro2A and SHSY5Y) as models. Biochemical, molecular and cellular biology techniques were used for this study.

Results: Domain analysis shows that RBM45 contains 3 RNA-recognition motifs, sharing similar structural elements with TDP-43 and FUS. Immunolocalization showed RBM45 is a nuclear protein, and the disruption of its nuclear localization signal (NLS) mis-localizes RBM45 to the cytoplasm. Reciprocal

co-immunoprecipitation assays indicated physical interactions between RBM45 and TDP-43 and FUS. RBM45 domains responsible for these protein–protein interactions were determined using truncation analysis. We also discovered that RBM45 can self-aggregate and determined the domain required for self-aggregation. The sequestering effect of RBM45 aggregation was examined. To identify the RNAs bound and regulated by RBM45, we have developed a novel CLIP-seq (crosslinking and immunoprecipitation-coupled RNA-seq) approach. The CLIP-seq data and the genes jointly regulated by RBM45, TDP-43 and FUS will be presented.

Discussion: Our results demonstrate that RBM45 is a new RNA-binding protein implicated in ALS and FTLD. We propose that RBM45 is a nuclear protein that functions in RNA splicing and transport. Nuclear import defects and environmental stress mis-localize RBM45 to the cytoplasm, where cytoplasmic aggregation can occur. The accumulation of cytoplasmic RBM45 disrupts normal RNA processing, contributing to cell death. Future mechanistic studies of RBM45 are warranted to further define the roles of RBM45 inneurodegeneration, which will broaden therapeutic options for ALS and FTLD.

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P270 THE RNA-BINDING PROTEIN RBM45 ASSOCIATES WITH NUCLEAR STRESS BODIES DURING CELLULAR STRESS EVENTS

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Keywords: RBM45, RNA binding proteins, nuclear stress bodies

Background: The RNA-binding protein RBM45 is a component of the inclusion bodies found in neurons and glia in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and Alzheimer's disease (AD). In neurons in human neurodegenerative disease post-mortem tissue, RBM45 also exhibits a speckled nuclear staining pattern that is independent of inclusion bodies. This staining pattern is suggestive of several subnuclear structures, including nuclear speckles, nuclear gems, Cajal bodies, and nuclear stress bodies. Which of these contain RBM45 is unclear.

Objectives: The purpose of this study was to investigate the potential association of RBM45 with a variety of subnuclear structures, and determine the biological factors governing the assembly of RBM45 into granules within the nucleus.

Methods: We examined subnuclear structures via immunocytochemistry using well-characterized markers of these structures, including nuclear speckles (SC35), Cajal bodies (coilin), nuclear gems (SMN), and nuclear stress bodies (HSF1 and SAM68). To investigate potential mechanisms of RBM45 inclusion formation, we also examined stress granules using TIA-1 and TIAR. The presence of absence of RBM45 within each subnuclear structure was determined using a combined digital deconvolution/quantitative immunocolocalization approach. Automated image analysis was also used to determine the number and size of RBM45-positive granules within the nucleus of cells. These methods were supplemented with co-immunoprecipitation experiments to identify potential RBM45-interacting proteins.

Results: We found that RBM45 does not co-localize with markers of nuclear speckles (SC35), Cajal bodies (coilin), or nuclear gems (SMN) and likewise is not a component of cytoplasmic stress granules. In untreated cells, RBM45 was diffusely localized throughout the nucleus, with few granules present. In contrast, when cells were subjected to heat shock or genotoxic stress, the number and size of RBM45-positive granules significantly increased ($p < 0.05$). We identified significant colocalization of RBM45 and the nuclear stress body-associated proteins HSF1 and SAM68 in stressed cells ($p < 0.05$). RBM45 did not exhibit significant colocalization with any of the other markers used ($p > 0.05$). Co-immunoprecipitation experiments demonstrated that RBM45 could be pulled down using anti-HSF1 antibodies and that HSF1 could be pulled down using anti-RBM45 antibodies.

Discussion: Collectively, these results demonstrate that RBM45 is a new component of nuclear stress bodies and define a role for RBM45 in the cellular response to stress. The incorporation of RBM45 into these structures may have important implications for the process of RBM45 inclusion formation. The results likewise identify a novel biological process as a potential therapeutic target for neurodegenerative disorders such as ALS, FTLD, and AD.

P271 CHARACTERIZATION OF THE ROLE OF SIGMA RECEPTOR 1 (SIGMAR1) IN MOTONEURON FUNCTION AND DISEASE

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Keywords: sigmar1, MAMs, motoneuron

Background: Mitochondria-associated membranes (MAMs) allow a bidirectional cross-talk between endoplasmic reticulum (ER) and mitochondria. MAMs have been shown to be involved in the control of lipid biosynthesis, mitochondrial division, calcium signaling and in the dynamics of the two organelles (1). Sigmar1, which is highly expressed in motoneurons and localized in large cholinergic postsynaptic densities (2), was previously shown to play a role in MAMs (3). Importantly, previous studies revealed that a recessive mutation in *SIGMAR1* is associated with a juvenile form of amyotrophic lateral sclerosis (ALS) (4). Sigmar1 localization and potentially its function was also shown to be affected in ALS models (5) underscoring the importance of MAMs for motoneuron function. While these data strongly support an involvement of Sigmar1 in MND, the mechanisms linking a loss and/or inactivation of Sigmar1 to motoneuron dysfunction remain to be clarified.

Objectives: Our project aims at characterizing the role of Sigmar1 in the function of motoneurons both *in vitro* and *in vivo,* in order to understand whether and how its deficiency contributes to the development of neurodegenerative disorders.

Methods and results: To this end, we performed *in vitro* experiments using primary motoneuron cultures in which Sigmar1 was either pharmacologically blocked (via specific antagonists) or genetically deleted (*Sigmar1*[Gt(OST422756)Lex] mice). Using these paradigms, we showed that blockade of Sigmar1 was sufficient to induce death of a subpopulation of motoneurons. Interestingly we showed that motoneuron death was linked to dysregulation of calcium pathways associated with ER and mitochondria dysfunction. In addition, our preliminary *in vivo* histological data confirmed that *Sigmar1*^{-/-} mice exhibit molecular and cellular defects in both motoneurons and muscles. Those defects may underlie previously described motor disabilities observed in this model (2).

Discussion and conclusion: Our results so far confirm the critical role of Sigmar1 in motoneuron function, and provide insight into the pathophysiological mechanisms involved in the development of ALS (3). We anticipate that further characterization of cellular pathways linked to Sigmar1 inhibition and/or loss will contribute to the identification of new therapeutic targets in the context of ALS.

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P272 METABOLOMIC APPROACH ON AN *IN VITRO* MODEL OF AMYOTROPHIC LATERAL SCLEROSIS, A CO-CULTURE OF ASTROCYTES AND MOTOR NEURONS EXPOSED TO OXIDATIVE STRESS

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Keywords: metabolomics, co-culture, oxidative stress

Background: Although genetic and environmental factors have been associated with amyotrophic lateral sclerosis (ALS) and despite evidences for implication of oxidative stress and excitotoxicity, pathophysiology of ALS remains unknown in the majority of cases (1). Preliminary results from our laboratory on cerebrospinal fluid components of ALS patients indicate a potential modification of energetic substrates consumed by the central nervous system (2).

Objectives: In order to improve the knowledge of the pathophysiology of this disease and associated metabolic alterations, we developed a metabolomic approach on a cellular model-reproducing genetic (in particular mutation in *SOD1* gene) and "environmental" (oxidative stress) conditions of ALS.

Methods: A co-culture model of mouse astrocytes (C8-D1A cell line) and motor neurons (NSC-34 cell lines) expressing or not human SOD1 protein wild-type or G93C mutant were exposed to oxidative stress (hydrogen peroxide and menadione). Oxidative stress status was characterized by ROS measurement, 8-hydroxy-2'-deoxyguanosine and malondialdehyde measurement, oxidant and antioxidant status evaluation and measurement of enzymes activities such as glutathione peroxidase, glutathione reductase and superoxide dismutase and evaluation of the ratio of reduced glutathione on oxidized glutathione (GSSG/GSH).

The intra- and extracellular media were collected at 3 times post-stress (for 48h) and analyzed by a multimodal analytical approach (liquid chromatography coupled to high-resolution mass spectrometry, proton nuclear magnetic resonance, and gas chromatography coupled to mass spectrometry). The evolution of metabolites concentrations during oxidative stress was studied in parallel to assessment of viability.

Results: Once the optimal conditions for the co-culture and the sample pretreatment procedure were established, we identified various metabolites such as amino acids, organic acids, and sugars. Preliminary analysis of results has showed about ten metabolites whose concentrations varied significantly during exposure to oxidative stress. In addition to increased levels of antioxidant molecules (vitamin C and taurine) in stressed co-cultures, we noted a decrease in their global metabolism, including reduced consumption of glucose and many amino acids.

Discussion and conclusion: Preliminary results from this study revealed the feasibility of a metabolomic multimodal approach on a cellular model of ALS. These data could highlight metabolic pathways altered in ALS and could open perspective of functional, genomic, and transcriptomic approaches.

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P273   ALS ASTROCYTES KILL MOTOR NEURONS VIA LIGATION OF DEATH RECEPTOR 6 BY A FRAGMENT OF N-APP/APLP1

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Keywords: non-cell autonomous death, astrocytes, death receptor 6

Mutations in superoxide dismutase-1 (SOD1) cause a form of amyotrophic lateral sclerosis (ALS). Previously, we have shown that primary or embryonic stem cell (ES)-derived MNs are killed by mutant SOD1-expressing astrocytes or by their conditioned medium (CM). Here, we show that the deleterious effects of mutant astrocytes are due to a toxic activity and not to lack of beneficial effects on MNs. This toxic activity is mediated by a negatively charged protein of ~5–30 kDa, which we surmised might be a ligand to a MN cell surface transduction protein.

Based on this premise, using liquid chromatography-mass spectrometry analysis, we found a list of 121 potential ligands selectively enriched in, or unique to, the anionic fraction of the toxic mutant astrocyte CM. To identify the extracellular ligand mediating the astrocyte dead signal within this list and its associated cell surface transducer, we decided to adopt a non-bias genome-wide approach. For this, purified ES-MNs were exposed to the ALS astrocyte CM for 72 h and then analyzed using RNA sequencing. From these gene expression profiling data, differential protein activity was inferred through a regulatory network-based approach that we have recently developed.

We found significant changes in the activity of 84 membrane proteins (FDR < 0.01). To narrow-down this list of candidate receptors, we combined the inferred activity with previous knowledge on protein–protein interaction catalogued in the STRING V9.1 database. Specifically, we selected those putative cell surface transduction proteins whose interaction with any of the 121 mutant SOD1 astrocyte-released proteins has been reported with high confidence (STRING score, > 850). We ended up with 5 putative receptor/ligand couples (FDR, < 0.2) that we have tested systematically in our co-culture models using a combination or immunological and genetic strategies.

We found that the neutralization or genetic ablation of the second most activated receptor, death receptor 6 (DR6, also known as TNFRSF21), in the MN compartment was fully protective. In agreement, in the astrocytes, the mirror neutralization or ablation of amyloid beta precursor protein (APP) or of amyloid precursor-like protein 1 (APLP1), two potential ligands of this orphan receptor, also completely reversed MN death. It was also noted that inhibitors of beta-secretase (BACE1) were protective, whereas a recombinant of the E1 domain of N-terminal APP was toxic to MNs in a DR6-dependent manner. Supporting the relevance of these findings to ALS is our observation that astrocytes from sporadic ALS patients also kill MNs by a DR6/APP/APLP1-dependent mechanism.

Thus, the present study not only reports on a new disease mechanism that rests on a deleterious molecular interaction between MNs and glial cells, but also opens new promising therapeutic avenues for this incurable disease.

P274   UNRAVELING THE MOLECULAR DEATH CASCADE TAKING PLACE IN MOTOR NEURONS IN RESPONSE TO ALS-LINKED ASTROCYTE TOXICITY

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Keywords: non-cell autonomous death, NFkB, RIPK1

Background: Mutations in superoxide dismutase-1 (SOD1) is a cause of amyotrophic lateral sclerosis (ALS), which is characterized by the death of large cholinergic motor neurons (MNs) in the spinal cord. Transgenic mice expressing mutant SOD1 develop a phenotype, which emulates the clinical and pathological hallmarks of ALS, including the early retraction of nerve terminals from the neuromuscular junction. However, the molecular mechanisms that lead to MN death remain unknown. We have reported that wild-type primary or embryonic stem cell-derived spinal MNs (ES-MNs) show significant death upon exposure to astrocytes expressing mutant SOD1 or their conditioned medium. We have also shown that the death response to mutant SOD1-expressing astrocytes is selective to MNs in a mixed culture model.

Objectives: Here, our objective is to elucidate the molecular death cascade which is specifically induced in MNs.

Results: We found that purified ES-MNs exposed to mutant astrocyte-conditioned medium (ACM) are a powerful model system to unravel the molecular mechanism(s) underlying this ALS-linked astrocyte neurotoxicity using microarray analysis. Using this model system, we have found 72 h of exposure to mutant ACM to be the point-of-no-return for MNs. Following this finding, we have completed gene array analyses on MNs after 12, 24, 48, and 72 h of exposure to mutant ACM to decipher early transcriptional alterations. In addition to common microarray analyses to assess genes that are differentially expressed, we have utilized an innovative reverse-engineering method. Here, gene expression data are integrated on a signaling interactome to infer the differential activity of signaling proteins. Using this approach, we have found NF-kappaB transcription factor activity at 12 and 24 h to be up-regulated in MNs exposed to mutant ACM. In parallel, by using cell death profiling and pharmacological approaches, we have found that RIP1, which is instrumental to the induction of necroptosis through its interaction with RIP3, is independently necessary for MN death induction. Indeed, inhibition of RIP1 kinase activity by the pharmacologic agent necrostatin-1 (Nec-1), or viral sh-RNA mediated knockdown of RIP1 transcript protects MNs from the death-mediated mutant SOD1 astrocyte-mediated MN death.

We are in the process of determining whether NFKB and RIP1, which are both independently necessary for MN death induction, function in the same pathway or distinct pathways.

Discussion: In further understanding the molecular pathways underlying MN death induction in our model, we anticipate that the generated information will be of critical importance for the development of effective neuroprotective therapies for familial ALS linked to mutant SOD1, as well as for sporadic ALS, as we and others are reporting that human sporadic ALS astrocytes are also toxic to MNs.

P275 ABNORMAL GLUTAMATE RELEASE INDUCED BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN EXPERIMENTAL ALS

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Keywords: excitotoxicity, glutamate release, presynaptic mGlu1/mGlu5 receptors

Background: The mechanisms of neuronal death in ALS are still largely obscure. It is well known that glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons. Although astrocyte uptake is thought to be a major cause (1), according to our previous studies, we have suggested that the high levels of synaptic Glu are also due to abnormal release of the amino acid (2).

Objectives: To investigate the modulation of Glu release by Group I metabotropic glutamate auto-receptors (mGluR1 and mGluR5) in spinal cord of SOD1^{G93A} mice.

Methods: SOD1^{G93A} mice expressing high-copy number of mutant human SOD1 with a Gly93Ala substitution and wild-type human SOD1-expressing control mice were used (3). Spinal cord synaptosomes were purified by homogenization and separation on discontinuous Percoll® gradient and used for glutamate release, measured by labeling synaptosomes with [^{3}H]D-Aspartate ([^{3}H]D-Asp), and confocal microscopy experiments (4),Western blot protein determination (5), cytosolic IP3 quantification, was measured using a commercial assay kit.

Results: Exposure of spinal cord synaptosomes to increasing concentrations of 3,5-DHPG, a mGluR1/5 agonist, produced distinct effects in SOD1^{G93A} and control mice: concentration above 0.3 μM stimulated the basal release of [^{3}H]D-Asp, both in control and in SOD1^{G93A} mice. At variance, concentrations of 3,5-DHPG equal to or lower than 0.3 μM increased [^{3}H]D-Asp release in SOD1^{G93A} mice only. Experiments with selective mGluR1 or mGluR5 antagonists indicated that the

3,5-DHPG effects involved both mGluR1 and mGluR5 activation. According to release experiments, high 3,5-DHPG concentrations increased IP3 in both mouse strains, whereas low 3,5-DHPG induced IP3 formation in SOD1^{G93A} mice only. 3,5-DHPG elicited [^{3}H]D-Asp exocytotic release involving intra-terminal Ca^{2+} release through IP3-sensitive channels. Confocal microscopy indicated the co-existence of both receptors in the same glutamatergic nerve terminal and Western blot analysis showed higher expression of mGluR5 in SOD1^{G93A} mice.

Discussion and conclusion: We can conclude that the activation of both mGluR1 and mGluR5, sited at spinal cord glutamatergic nerve terminals, produces abnormal Glu release in SOD1^{G93A}mice, suggesting their involvement in determining the high extracellular Glu levels present in ALS. These results would prompt for new pharmacological approaches.

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P276 MOLECULAR MECHANISM UNDERLYING EXCESSIVE AND PRECOCIOUS GLUTAMATE RELEASE IN THE SPINAL CORD OF THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: glutamate release, excitotoxicity, synaptic proteins

Background: Selective and progressive neurodegeneration of upper and lower motoneurons is a typical hallmark of amyotrophic lateral sclerosis (ALS). Motoneuron cell death in ALS has been ascribed to multiple causes, including glutamate (Glu)-mediated excitotoxicity. Impaired astrocytic Glu transport was suggested as the reason of high synaptic Glu levels (1); however, other causes may concur in elevating extracellular Glu in ALS. Our previous studies highlighted the presence of abnormal Glu release induced by activation of heterologous GABA and glycine transporters expressed at Glu-releasing nerve terminals in the spinal cord of SOD1^{G93A} mice (2–4).

Objectives: This study was aimed to verify the occurrence of excessive Glu exocytosis in experimental ALS as a possible cause of the disease.

Methods: Pre-symptomatic and symptomatic SOD1[G93A] mice were used. Nerve terminals (synaptosomes) were obtained from the spinal cord of control and SOD1[G93A] mice (5), and utilized for release experiments by exploiting the superfusion technique. Cytosolic calcium determination, confocal microscopy and Western blot experiments were performed to investigate the expression and the activation state of synaptic proteins.

Results: Both the spontaneous and the stimulus-evoked exocytotic Glu release was increased in SOD1[G93A] symptomatic and pre-symptomatic mice, compared to that in controls. Increased pre-synaptic Ca^{2+} levels, over-activation of calcium/calmodulin-dependent kinase-II and ERK/MAP kinases, as well as hyper-phosphorylation of synapsin-I, were determined at both symptomatic and pre-symptomatic clinical stages. The expression of several synaptic proteins implicated in neurotransmission does not show differences, except for the over-expression of synaptotagmin in SOD1[G93A] mice. Pre-synaptic hyper-phosphorylation at the inhibitory sites of GSK3 and accumulation of high molecular weight SNARE complexes was also measured. In line with these findings, release experiments suggested that the excessive Glu exocytosis involves the readily releasable pool of vesicles.

Discussion and conclusion: Our results indicate a dysregulation of glutamate exocytosis in the spinal cord of symptomatic and pre-symptomatic SOD1[G93A] mice. This event was accompanied by marked changes in a number of pre-synaptic molecular mechanisms that lead to a significant augmentation of the readily releasable pool of vesicles and determine a higher probability of vesicles to fuse. Synaptic alterations, found to be present also in a pre-symptomatic stage, could represent a key feature in the early phase of ALS, thus playing a role in the etiopathogenesis of the disease.

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P277   REDUCE GLUTAMATE UPTAKE IN ACTIVATED ASTROCYTES IN SOD1(G93A) MICE AND ITS IMPLICATIONS ON ALS PATHOGENESIS

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Keywords: neurotrophic factors, glutamate, astrocyte activation

Background: Astrocyte activation occurs in response to central nervous system (CNS) insult and is considered a double-edged sword in many pathological conditions. We propose that reduced glutamatergic and trophic response of astrocytes to activation may, over time, lead to accumulative CNS damage, thus facilitating neurodegeneration.

Results: We found that astrocytes derived from the SOD1[G93A] ALS mouse model exhibit a reduced glutamatergic and trophic response to specific activations compared to their wild-type counterparts. Wild-type astrocytes exhibited a robust response when activated with lipopolysaccharide (LPS), G5 or treated with ceftriaxone in many parameters evaluated. These parameters include increased expression of GLT-1 and GLAST the two major astrocytic glutamate transporters, accompanied by a marked increase in the astrocytic glutamate clearance and up-regulation of neurotrophic factor expression. However, not only do un-treated SOD1[G93A] astrocytes take up glutamate less efficiently, but in response to activation they show no further increase in any of the glutamatergic parameters evaluated. Furthermore, activation of wild-type astrocytes, but not SOD1[G93A] astrocytes, improved their ability to protect the motor neuron cell line NSC-34 from glutamate-induced excitotoxicity. All experiments were performed at least in three separate biological replications in triplicates.

Conclusion: Our data indicate that altered astrocyte activation may well be pivotal to the pathogenesis of ALS.

P278   E6-AP PROMOTES SOD1 PROTEIN DEGRADATION AND SUPPRESSES MUTANT SOD1 TOXICITY

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Keywords: protein misfolding, SOD1, degradation

Background: Recent studies indicated that E6-AP, a homologous to E6-AP C terminus (HECT)-type E3 ubiquitin ligase, has a role in cellular protein quality control system and is implicated in the degradation of expanded polyglutamine proteins. However, the roles of E6-AP in motor neuron diseases have not been explored.

Objectives: The aim of this study is to examine the role of E6-AP in mutant SOD1-mediated ALS.

Methods: The expression levels and localization of E6-AP proteins were examined in the spinal cord of various mutant SOD1 mice. Using cultured cells, protein interaction between SOD1 and E6-AP and the ubiquitination and degradation of SOD1 protein in the presence of E6-AP were analyzed. Further, the role of E6-AP in ameliorating mutant SOD1-mediated cellular toxicity was analyzed in cultured cells with overexpression of molecular chaperone, Hsp70.

Results: Endogenous E6-AP was depleted from the motor neuron nuclei of SOD1-ALS mouse models prior to neurodegeneration. E6-AP co-immunoprecipitates with the SOD1 protein and is predominantly mislocalized in mutant SOD1-

containing inclusion bodies. Overexpression of E6-AP increases the ubiquitination and facilitates degradation of SOD1 proteins. Finally, overexpression of E6-AP suppresses the aggregation and cell death mediated by mutated SOD1 proteins, and cellular protective effect is more prominent when E6-AP is overexpressed along with Hsp70.

Discussion and conclusion: These data suggest that enhancing the activity of E6-AP ubiquitin ligase might be a viable therapeutic strategy to eliminate mutant SOD1-mediated toxicity in ALS.

P279   CYSTATIN C PROTECTED NEURONAL CELLS AGAINST MUTANT COPPER-ZINC SUPEROXIDE DISMUTASE-MEDIATED TOXICITY *IN VITRO*

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Keywords: cystatin C, superoxide dismutase 1, autophagy

Background: Recent studies have demonstrated Cystatin C (CysC), an endogenous cysteine protease inhibitor, plays neuroprotective roles in Alzheimer's and Parkinson's diseases. In sporadic ALS, CysC is a major component of Bunina bodies and decreased in the patients' cerebrospinal fluid. However, it remains unclear whether CysC has a protective role in ALS.

Objectives: The aim of this study is to examine the potential of CysC as a novel neuroprotective agent in ALS.

Methods: We added recombinant human CysC to neuro2a cells transiently expressing mutant SOD1. The cell viability was measured using MTS assay. We also examined the neuroprotective effect of CysC using the primary neuron-glia mix culture derived from Hb9:GFP/SOD1[G85R] double transgenic mouse embryo. Furthermore, to investigate the neuroprotective mechanism of CysC, we examined the possible involvement of autophagy pathway.

Results: Exogenously added CysC protected neuro2a cells against mutant SOD1-mediated cytotoxicity in a dose-dependent manner. Intracellular aggregates of mutant SOD1 were remarkably decreased in the CysC-treated cells. CysC was also protective for primary cultured neurons. Moreover, the level of LC3-II was increased in the CysC-treated cells and 3-methyl adenine treatment clearly inhibited the protective effect of CysC, suggesting that the induction of autophagy was required for neuroprotection by CysC.

Discussion and conclusion: These findings suggest that CysC is a novel therapeutic candidate that can protect neurons against mutant SOD1 toxicity through induction of autophagy-lysosomal pathway.

P280   INCREASED AMPK ACTIVITY AND DOWNREGULATION OF HSP70 EXPRESSION DECREASE THE LIFESPAN OF SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: AMPK, Hsp70, high-fat diet

Metabolic abnormalities are observed in amyotrophic lateral sclerosis (ALS). The molecular mechanisms of the abnormal metabolism remain unclear. AMP-activated protein kinase (AMPK) is essential for neuronal integrity and survival. AMPK is activated by metabolic stresses, including ischemia, hypoxia and caloric restriction (CR), while hyperglycaemia, feeding and a high-fat diet (HFD) inhibit AMPK activation. In this study, we found that AMPK activity increased and heat shock protein-70 (Hsp70) was downregulated in the spinal cord of SOD1[G93A] transgenic mice compared to SOD1[WT] controls. Furthermore, we showed that CR increased AMPK activity, downregulated Hsp70 expression, aggravated the loss of motor neurons, hastened disease onset and reduced the lifespan of SOD1[G93A] transgenic mice compared to ad libitum (AL) SOD1[G93A] controls. In contrast, HFD inhibited AMPK activity and motor neuron loss, upregulated Hsp70 expression, delayed disease onset and extended survival in these mice. These results suggest that AMPK activity plays a negative role in the survival of motor neurons, possibly through a novel mechanism involving Hsp70 downregulation, such that treatments causing inhibition of AMPK slow the progression of motor neuron disease in this mouse model.

P281   PHENOTYPIC DISCOVERY AND CHARACTERIZATION OF NEUROPROTECTIVE COMPOUNDS RELEVANT TO ALS

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Keywords: drug discovery, high content screening, in vitro pharmacology

Disease modalities such as Parkinson's, ALS and Alzheimer's represent complex neurological disorders where certain neuronal populations decline via only partially understood mechanisms that frequently involve neuro-inflammation. In order to identify new and disease-relevant, neuroprotective compounds for ALS, we built a screenable phenotypic assay that re-builds aspects of the disease in a microtiter plate. We modelled the non-cell autonomous nature of ALS by integrating stem cell-derived motor neurons, astrocytes and activated microglia into the screening set up. As primary biomarker of ALS, we used degeneration of motor neurons and identified a small number of neuroprotective compounds in a screen of 11,000 compounds.

Through diverse secondary assays, we showed that these hit compounds act through multiple mechanisms, including the inhibition of microglial activation, directed protection of neurons from nitric-oxide-induced degeneration, and glia-specific activation of genes controlled by Nrf2, a transcription factor previously shown to be effective in the SOD1^{G93A} mouse model.

The modular nature of the presented phenotypic assay allows for integration of both additional stress paradigms and cells relevant to specific types of neurodegenerative disease. Further integration of chemical biology, proteomics, and network biology tools will allow unravelling targets and molecular mechanisms of action. Thus, hit compounds identified and characterized in these ways may represent ideal starting points for the development of new drugs to treat various neurodegenerative diseases.

P282   VITAMIN D CONFERS PROTECTION TO MOTONEURONS AND IS A PROGNOSTIC FACTOR OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: vitamin D, motoneuron culture, survival

Background: Vitamin D (VD) is a potent secosteroid hormone with diverse biological functions that include protection against neuronal damage. The detrimental consequences of VD dietary deficiency have been documented in several neurodegenerative diseases. We reported in 2012 that ALS patients with severe VD deficiency had a 6 times more rapid worsening than those with normal levels.

Objectives: We assessed the effect of VD on rat purified motoneurons *in vitro*. We also actualized data from our ALS cohort, for overall survival.

Methods: We studied the effect of 1,25(OH)$_2$ D$_3$ (the biologically active hormone),vitamin D2 (VD2) and vitamin D3 (VD3) on the electrophysiological properties and survival features of purified motoneurons (MNs) from E12.5 mice embryos. Purified MNs were obtained using iodixanol density gradient centrifugation and then plated on poly-ornithine/laminin-treated wells.

Results: The addition of 1,25(OH)$_2$D$_3$ in the culture medium increased intensity of nuclear staining of the VD receptor (VDR), while addition of VD$_2$ and VD$_3$ did not. In culture, 1,25(OH)$_2$D$_3$ significantly improved MN survival by 45% (100nM, p < 0.001), while VD2 and VD3 did not. Interestingly, the effect of 1,25(OH)$_2$D$_3$ was abolished when neurotrophic factors were removed from the culture medium. As accumulating evidence suggests that the MN-restricted Fas death pathway may contribute to the degenerative process in ALS, MNs were cultured for 24 h before being treated for 48 h with soluble Fas Ligand in combination of either VD$_2$, D$_3$ or 1,25(OH)$_2$D$_3$. After this delay, 1,25(OH)$_2$D$_3$ completely rescued MNs from Fas-induced death, while neither VD$_2$ nor D$_3$ influenced cell survival. *In vitro*, 1,25(OH)$_2$D$_3$, did not modify electrical properties of MNs.

In parallel, we determined the outcome of our initial group of 74 ALS patients with VD level measurement. These patients were included in a follow-up, until death, between 2010 and 2011. By April 2013, 100% of the patients with severe VD deficiency (< 25 nmol/l) were dead, compared to 62% of those belonging to the group with VD deficiency (>25 and < 75) and only 25% of the patients with normal levels. This corresponded to a median survival of 22, 31 and 49 months for the groups of severe deficiency, deficiency and normal levels, respectively (p < 0.001).

Discussion: In ALS patients, VD deficiency is associated with a worse outcome when compared to patients with normal levels. *In vitro* experiments give results that are consistent with clinical data, showing that the active hormone 1,25(OH)$_2$D$_3$ promotes MN survival and protects against the Fas-induced apoptotic pathway. These concordant results may pave the way towards new therapeutic approaches in ALS.

P283   SODIUM AND CALCIUM OVERLOAD INDUCED BY VERATRIDINE IN NSC-34 CELLS: A NOVEL *IN VITRO* MODEL OF ALS TO EXPLORE NEW NEUROPROTECTIVE COMPOUNDS

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Keywords: NSC-34 cells, neuroprotection, TDP-43

Background: At present, therapeutic alternatives for amyotrophic lateral sclerosis (ALS) are scarce and controversial. Only riluzole delays death a few months. The aetiology of motorneuron vulnerability in this disease is being studied in NSC-34 cells that were created as hybrids of neuroblastoma cells and spinal motor neurons to find out the underlying mechanisms leading to the selective loss of motorneurons in ALS (1). One such mechanism is associated with TDP-43 (TARD DNA-binding protein 43) aggregates and Ca^{2+} buffering proteins driving more vulnerability of motoneurons degeneration.

Objectives: The main aim of this study proposes the overload of Na$^+$ and Ca^{2+} induced by veratridine (VTD) in NSC-34 cell line as an *in vitro* model of ALS. As an additional aim, we assessed whether the cytotoxic damage evoked by VTD could be altering the expression of TDP-43 and Ca^{2+} buffering proteins.

Methods: Cell viability was measured by MTT and cytosolic concentration of Ca^{2+} ([Ca^{2+}]$_c$) with the fluorescent probe Fura-2. Protein expression was measured using immunofluorescence.

Results: Cell viability was decreased by 79% (16 cells of 6 different experiments) after 48-h incubation with VTD

(100 μM). Riluzole produced 13% neuroprotection at 1 μM and 9% at 3 μM against VTD after 6 h pre-treatment (9 cells of 3 different experiments). ITH33/IQM9.21, a novel neuroprotective compound (2, 3), did not elicit neuroprotection at any of the concentrations tested (1, 3 and 10 μM) after 2 or 6 h pre-treatment (9 cells of 3 different experiments). Furthermore, VTD induced oscillations of the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_c$) in 46% of studied cells (141 cells of 7 different cultures). Riluzole abolished these oscillations of $[Ca^{2+}]_c$ in 93% of the cells (29 cells of 2 different cultures).

Discussion and conclusion: We propose VTD as a Na^+ and Ca^{2+} overload *in vitro* model to reproduce ALS in order to test new neuroprotective compounds.

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P284   IN VITRO EVIDENCE FOR THE THERAPEUTIC POTENTIAL OF MENSENCHYMAL STROMAL CELLS IN ALS

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Keywords: mesenchymal stromal cells, growth factors, inflammation

Background: Administration of mesenchymal stromal cells (MSC) has beneficial effects on motor function and survival in the SOD1G93A mouse model of ALS as well as in models of other neurological disorders.

Objectives: We intended to study the effect of the interaction between MSC and motor neurons and glial cells and to analyse the mechanisms underlying the protective effects of MSC *in vitro*.

Methods: MSC or MSC-conditioned medium (MSC CM) was added to embryonic primary motor neurons (derived from both non-transgenic and mutant SOD1[G93A] transgenic mice), NSC-34 cells and glial cells (astrocytes and microglia, derived from both non-transgenic and mutant SOD1[G93A] transgenic mice), and the effects against staurosporine-induced cell death were determined by immunocytochemistry and MTT assay. MSC CM-induced changes in mRNA expression of pro-and anti-inflammatory mediators in glial cells and on growth factor expression in motor neurons were quantified by real-time PCR.

Results: In primary motor neurons, NSC-34 cells and astrocytes, MSC CM attenuated staurosporine-induced apoptosis in a concentration-dependent manner. Studying MSC CM-induced expression of neurotrophic factors in astrocytes and NSC-34 cells, we found that glial cell line-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF) gene expression in astrocytes were significantly enhanced by MSC CM, with differential responses of nontransgenic and mutant astrocytes. Expression of vascular endothelial growth factor (VEGF) in NSC-34 cells was significantly upregulated upon MSC CM-treatment. MSC CM significantly reduced the expression of the cytokines TNFα and IL-6 and of iNOS in both transgenic and non-transgenic astrocytes. Gene expression of the neuroprotective chemokine Fractalkine (CX3CL1) was also upregulated in mutant SOD1[G93A] transgenic astrocytes by MSC CM treatment. Correspondingly, MSC CM increased the respective receptor, CX3CR1, in mutant SOD1[G93A] transgenic microglia.

Discussion and conclusion: Our data demonstrate that MSC modulate the motor neuronal and glial response to apoptosis and inflammation, and induce gene expression changes capable to contribute to neuroprotection. MSC therefore represent an interesting candidate for further preclinical and clinical evaluation in ALS.

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P285   EMBRYONIC STEM CELL-DERIVED MOTONEURON/MUSCLE FIBER CO-CULTURES: A MODEL SYSTEM FOR STUDYING AMYOTROPHIC LATERAL SCLEROSIS PATHOPHYSIOLOGY

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Keywords: Motor neuron, Stem cells, co-culture

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by motor neuron death. This devastating disease leads to paralysis of muscles of respiration and those of the limbs, eventually leading to death. Genetic defects in the copper/zinc superoxide dismutase (mSOD1) gene have been linked to familial ALS. One of the early features in the disease process is denervation of the neuromuscular junction (NMJ). It is not known whether denervation is simply a result of motor neuron (MN) death or whether denervation is one of the contributing variables causing the MNs to die. To address this question, we co-cultured wild-type mouse embryonic stem cell-derived motor neurons (ECSMNs) with chick myotubes to correlate NMJ formation, synaptic stability with the onset and time course of ECSMN cell death.

Methods: ESCMNs were derived from embryonic stem cells isolated from wild-type and transgenic mice expressing the G93A mutant form of humanSOD1. Both strains of mice expressed eGFP under the control of the motor neuron-specific Hb9 promoter. Stem cells were directed to differentiate into ESCMNs using retinoic acid and a smoothen agonist. Five days later, the ESCMNs were plated onto chick myotubes in a serum-free media that was supplemented with GDNF and CNTF. Growth factors were withdrawn 1 week later to promote neuromuscular synapse formation. ESCMNs

survival and NMJ morphology were quantified and correlated over the next 3–4 weeks.

Results and discussion: To date, our results indicate that there is no difference in the survival of WT and mSOD1 ESCMNs after the first week of co-culture in the presence of growth factors. After the withdrawal of growth factors, NMJs typically mature in WT ESCMN/myotube co-cultures over the next 2- to 3-week period. Maturation is characterized by an increase in size and complexity of the pre- and post synaptic structures at putative NMJs (ie, neurite, synaptic vesicles and acetylcholine receptors). Unlike WT ESCMN/myotube co-cultures, very few mature NMJs were observed in the mSOD1 ESCMNs co-cultures. The lack of mature NMJs was accompanied by a dramatic increase in mSOD1 ESCMN death compared to the WT ESCMN/myotube co-cultures. We are currently conducting detailed time course analysis to determine whether NMJs failed to form in the mSOD1 ESCMN/myotube co-cultures or whether they formed and later withdrew. If the latter is true, these results would support for the hypothesis that axonal withdrawal precedes, and may contribute to, MN cell death in ALS. Furthermore, it would indicate that the ESCMN/chick myotube co-culture is an ideal model system to study pathophysiologies associated with the NMJ.

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P286 ALTERED NEURAL FATE OF EPENDYMAL STEM PROGENITOR CELLS IN ALS G93A-SOD1 MOUSE MODEL: THE MICRORNA REGULATION

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Keywords: ependymal stem cells, microRNA

Background: Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by motoneuron degeneration. In ALS SOD1^{G93A} mice, motoneuron degeneration is associated with proliferation of ependymal stem progenitor cells (epSPCs), usually quiescent in the spinal cord (1) which does not lead to restoration. MicroRNAs (miRNAs) regulate gene expression at post-transcriptional level and coordinate self-renewal and stem cell differentiation in the spinal cord (2); hence, their evaluation is crucial to understand ALS neurodegenerative mechanisms.

Objective: We investigated: a) the *in vitro* neurogenesis of epSPCs isolated from the spinal cord of SOD1^{G93A} at asymptomatic and symptomatic phases of the disease; b) the expression of miRNAs specific for neural cell fate (miR-9 and miR-124a) and cell-cycle regulation (miR-19a and miR-19b) during neural differentiation of cultured epSPCs.

Methods: We characterized the capacity of SOD1^{G93A} and control epSPCs to proliferate and differentiate *in vitro* into the three neural cell lineages: neuron, astrocytes and oligodendrocytes. Neural cell lineages were identified by immunocytochemistry. The expression of miR-9, miR-124a, miR-19a, miR-19b, and their predicted mRNA targets were assessed using real-time PCR in undifferentiated and differentiated epSPCs.

Results: SOD1^{G93A} epSPCs produced neurospheres that differentiated into the three neural cell lineages: neurons were more numerous than astrocytes and smaller than those obtained from controls; astrocytes had an activated phenotype. miRNA analysis revealed that miR-9, miR-124a, miR-19a and miR-19b expression was altered during SOD1^{G93A} epSPC differentiation. A paired expression analysis of miRNAs and their predicted targets allowed the identification of a functional network composed of genes implicated in neural cell fate, cell maturation, cell migration and proliferation.

Discussion and conclusion: We demonstrated that SOD1^{G93A} epSPCs can differentiate into neural cells whose phenotypic alterations resemble those observed in ALS pathology. Analysis of the functional network suggested an involvement of miRNAs in epSPC neural fate, maturation, migration and proliferation through their effects on mRNA targets.

Our findings indicate that SOD1^{G93A} epSPCs are a valuable model recapitulating the pathogenetic steps of ALS and represent a new tool to investigate the mechanisms of neurodegeneration, their molecular control, and may serve to identify new targets for ALS therapy.

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P287   CLINICAL EFFICACY OF STEM CELLS IN ALS: CORRELATION WITH TROPHIC SUPPORT AND EXPERIMENTAL *IN VIVO* STUDY

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Keywords: mesenchymal stem cells, neuroprotection, trophic factor

Background: Bone marrow mesenchymal stem cell (MSCs) have the potential to modify the disease progression and show neuroprotective and paracrine capabilities in models of amyotrophic lateral sclerosis (ALS).

Objectives: We examined the clinical effects of autologous MSCs in ALS patients and conducted two experimental studies: the first study evaluated the trophic support by MSCs isolated from patients compared with clinical response; study 2 confirmed their efficacy through an *in vivo* model of ALS.

Methods: We enrolled 37 patients for stem cells therapy. MSCs were injected twice intrathecally at an interval of 1 month. Remnant MSCs after therapy were analysed to measure the protein levels of bFGF-2, SDF-1α, VEGF, IGF-1, BDNF, ANG, IL-4, IL-10 and TGF-b in culture supernatants. The individual levels were compared with the clinical response (responder vs non-responder), which were grouped after the closure of clinical trial. For the *in vivo* study, MSCs isolated from one of each group were transplanted into cisterna magna in SOD1 mice.

Results: There were no serious adverse events related to therapy in ALS patients. In the responder group, the levels of VEGF, ANG and TGF-b were higher, suggesting different trophic support between responder and non-responder groups. MSC-treated mice showed significant delayed symptom onset and decreased motor neuron loss compared to PBS-treated mice. Moreover, the mice group that received MSCs isolated from responder patient showed prolonged survival and attenuation of the functional decline of motor performance using rotarod test.

Discussion and conclusion: Although further study is needed, our research shows the possibility of selecting candidate patients for autologous MSCs therapy based on the specific characteristics of stem cells, especially trophic factors secreting capacity.

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P288   AMYOTROPHIC LATERAL SCLEROSIS: NEW THERAPEUTIC PERSPECTIVES OFFERED BY IPSC-DERIVED NEURAL STEM CELLS

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Keywords: neural stem cell, transplantation, iPSCs

Objectives: The primary aim of this project was to investigate the therapeutic potential of induced pluripotent stem cell (iPSC)-derived neural stem cell (NSC) transplantation in an established model of amyotrophic lateral sclerosis (ALS), SOD1^{G93A} mice.

Background: ALS is a fatal, incurable neurological disorder with a high social burden. Recently, the scientific community has paid increasing attention to iPSCs as a source of NSCs that can be used for both modeling and therapeutic transplantation strategies.

Methods: We generated iPSCs from healthy human skin fibroblasts through the overexpression of pluripotent-reprogramming factors after transient, non-viral transfection of episomal vectors. We differentiated iPSCs using an established protocol to promote neuronal fate. By FACS selection, we isolated a primitive NSC subpopulation, based on its high ALDH activity and low side scatter (ADLHhiSSClo).

This population was selected due to its capacity to proliferate and differentiate into the three neuroectodermal lineages, and to its high potential ability to reach the central nervous system (CNS). The phenotype of these cells was defined by morphological gene expression and protein profile analysis. iPSC-purified NSCs were administered by intrathecal or systemic intravenous injections into ALS mice, and neuropathological assays and functional tests were performed.

Results: We investigated ADLHhiSSClo NSCs ability to migrate to the CNS after minimally invasive injection and to engraft into the host spinal cord. We demonstrated that iPSC-derived NSCs transplantation significantly prolonged the lifespan of SOD1G93A mice. This approach also improved the disease phenotype in treated animals. Specifically, we observed a better survival of motor neurons, preservation of neuromuscular junctions, and improvement of motor capabilities, coordination and movement after transplantation.

Discussion and conclusions: These data suggest that iPSC-derived NSC transplantation represents a promising opportunity for effective cell-based therapy for ALS and other motor neuron diseases, highly suitable for clinical applications.

P289   ISOLATION OF PURE IPSC-DERIVED HUMAN MOTOR NEURONS BY A NOVEL P75/HB9 DOUBLE-SELECTION FACS PROCEDURE

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Keywords: IPS cells, FACS, qPCR

Background: The targeted differentiation of human-induced pluripotent stem cells (iPSCs) into ALS-relevant cell types offers unique opportunities for ALS disease modelling, drug testing and eventually cell replacement. Yet, the study of ALS-related phenotypes in iPS-derived cultures is hampered by high inter- and intra-individual variability of differentiating cultures.

Method: To prepare pure motor neuronal cultures, we generated iPS clones from ALS patients and healthy control subjects, optimized the differentiation protocol and developed a novel FACS selection procedure.

Results: The iPS-derived cultures contained motor neurons (HB9+: 14.6 ± 5.2 % of neurons) expressing markers of median motor neuron subtype (LHX3) or lateral motor neuron subtype (FOXP1). To purify these motor neurons from unwanted neural precursor and interneuron populations, we used a lentiviral reporter vector expressing RFP under control of a 3.6 kb-long minimal HB9 promoter in combination with a monoclonal antibody directed against a surface epitope of the low-affinity neurotrophin receptor p75. Using FACS, Hb9 (RFP)/p75 double-positive cells were isolated on a routine basis at a ratio of 0.16 ± 0.02 % of total. The FACS-isolated cells re-attached on culture dishes and developed large cell bodies with prominent neurites after several days *in vitro*. The bona fide motor neurons were exquisitely pure as judged from QPCR and immunofluorescence analyses.

Discussion: We anticipate that pure cultures of IPS-derived human motor neurons will pave the way to indepth studies of ALS-relevant disease phenotypes and experimental therapies.

P290   ENDOGENOUS STEM CELL MOBILIZATION IN A MOUSE MODEL OF ALS

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Keywords: hematopoietic stem cell, Neulasta, stem cell therapy

Background: One of the most common motor neuron diseases is the well-known disease ALS, which nowadays lacks an appropriate therapy. The challenge is the search for new therapeutic approaches. Recently, cell therapy has risen as a potential candidate for the treatment of neurodegenerative diseases (1). In this study, we propose the use of Pegfilgramstin, a pegylated form of the Granulocyte Colony Stimulation Factor pegylated analog Filgrastim, which is commercially available from Amgen with the name of Neulasta® and is usually used in human clinics.

Objectives: Our main aim is to study the effect of Pegfilgramstin (Neulasta®) in transgenic SOD1^{G93A} mice to analyze the possible hematopoietic stem cell (HSC) mobilization response.

Methods: Transgenic SOD1^{G93A} mice were treated subcutaneously with Pegfilgramstin, once at week beginning at the age of 70 days. At 75, 90, 105 days and endpoint stage, blood samples were extracted from tail vein and HSC, myeloid precursor cells (MPC), lymphoid precursor cells (LPC), monocytes and lymphocyte cells were quantified by flow cytometry and identified according to antigenic expression pattern. Behavioural tests and survival of the animals were monitored. Transcriptional expression of neurodegeneration markers was analyzed using real-time PCR.

Results: Animals treated with Neulasta showed a significant increase in survival rate. Furthermore, a slower decline of motor functions was observed in mice treated with Neulasta. Flow cytometry revealed that HSC, LPC and MPC were mobilized by Neulasta, and this effect was prolonged over time. At the endpoint stage, a significant reduction in the relative number of HSC was observed in treated animals. The transcriptional level of *Impa1* and *Nnt* showed a down-regulation. Additionally, the transcript levels of *Col19a1* and *Mef2c* were down-regulated. No statistically significant differences were observed, under treatment, in the transcriptional levels of Pax7 and myogenic regulatory factors, although a robust down-regulation of *Chrma1* and *RRad* transcriptional expression was observed. Previous studies support these results (2,3).

Discussion: Mobilization of endogenous HSC by Neulasta demonstrated a beneficial effect on locomotor performance and prolongs the survival of transgenic SOD1[G93A] mice. Over time, Neulasta treatments increased HSCs inperipheral blood. Moreover, under Neulasta treatment, the NMJ stabilization and muscle metabolic restoration can be improved in the animals. These results suggested that mobilizing stem cells, facilitating their transformation and assisting in their recruitment by damaged tissue could be a potential therapeutic approach. Further studies will be needed to determinate its molecular mechanism.

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P291   SCF-ACTIVATED BONE MARROW TRANSPLANTATION IN ALS MODEL MICE

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Keywords: stem cell factor, bone marrow-derived cell, microglia

Background: Transplantation of bone marrow derived-cells or hematopoietic stem cells from wild-type mice was effective in an ALS mouse model. In addition, growth or differentiation factors were reported to protect motor neurons. However, clinical trial of bone marrow transplantation (BMT) or supplement of differentiation factors has shown no successful gain. Additional development is required for the treatment of ALS patients. We hypothesis that a combination therapy of BMT and differentiation factors has the possibility to enhance therapeutic effect.

Objectives: To clarify that the stimulation of stem cell factor (SCF) or FMS-like tyrosine kinase 3(flt3) enhances the therapeutic effect of BMT in hSOD1[G93A] transgenic mice.

Methods: We gave BMT to 8-week hSOD1[G93A] transgenic mouse after pre-incubation of bone marrow cells from wild-type mouse with SCF, flt3 or nothing for 12 h. Following transplantation, rotarod motor function test was performed and survival rate was checked, every week. In addition, we analyzed the appearance and the character of bone marrow-derived cell in spinal cord using bone marrow cells from GFP transgenic mice.

Results: Motor function and survival rate were ameliorated in the SCF-stimulated BMT group (SCF-BMT) more than in the flt3-stimulated BMT group (flt3-BMT) and in non-stimulated BMT group (WT-BMT). During this time, many bone marrow-derived cells migrated in spinal cord; this number was remarkably increased in SCF-BMT compared to that in flt3-BMT and in WT-BMT. The majority of bone marrow-derived cells in spinal cord expressed Iba1 known as a microglia marker. Furthermore in the SCF-BMT group, cells expressed glutamate transporter 1(GLT-1).

Discussion: The combination therapy of SCF pre-incubation and bone marrow transplantation improves the therapeutic effect of BMT. The effect is likely caused by the change of migrated bone marrow-derived microglia to neuroprotective cells. This combination therapy is superior to simple BMT treatment, and has a high potential of a new therapy for ALS patients.

P292   INTRAMUSCULAR TRANSPLANTION OF MUSCLE PROGENITOR CELLS THAT SECRET NEUROTROPHIC FACTORS SIGNIFICANTLY DELAY THE SYMPTOMS AND INCREASE THE LIFESPAN OF MSOD1 MICE

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Keywords: neurotrophic factors, muscle progenitor cells, retrograde transport

Introduction: Neurotrophic factors (NTFs) preserve and protect motor neuron in ALS models. However, all of the clinical studies with administration of NTFs in ALS patients failed. We have developed muscle progenitor cell (MPCs) populations expressing BDNF, GDNF, VEGF or IGF-1, (MPC-NTFs). Combined conditioned media collected from the cells rescued motor neurons cell line (NSC-34) from various insults. Furthermore, MPC-NTF transplantation enhanced the regeneration of rat sciatic nerves after injury. Here we examined the retrograde transport of NTFs along motor neuron axons and tested whether MPC-NTFs transplanted into muscles can improve the symptoms and survival of SOD1 mice.

Results: A mixture of MPC populations each expressing one of the four NTFs was transplanted into the hind legs of SOD1 mice on days 90, 104 and 118 of life. We found a significant delay of the symptoms (up to 30 days in the male) and extension of lifespan (12 days in the males and 18 days in females). However, transplantation of MPC alone or MPC overexpressing just GDNF did not elicit any improvement. The results suggest a synergistic effect of the transplantation of MPCs expression several NTFs. In order to study the possible synergistic effect of the mixture of MPC-NTFs on a signal transduction pathway; we focused on the PI3K-AKT motor neuron survival pathway. We found that the supernatant of a mixture of condition media from MPC populations expressing NTFs increases the phosphorylated AKT by 6- to 8-fold compare to MPC expressing a single NTF.

Discussion and conclusion: Here, we have built a novel powerful strategy enabling a stable, long-term administration of four NTFs factors cocktail. Since intramuscular inoculated muscle progenitor cells participate in the formation of post-mitotic multinucleated fibers, this route of administration of genetically manipulated MPCs results in a stable, long-term expression of the four NTFs. The constant and continuous

releases of the critical NTFs from the muscle fibers through the neuromuscular junction into the motor neuron system, and the retrograde transport to the cell bodies in spinal cords probably inhibit cell death pathways. We hope that our study will lead to a novel strategy to slow the progress and alleviate the symptoms of ALS, and extend the life expectancy and quality of affected patients.

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P293   PERSONALIZING ALS TREATMENT: DIRECT AND SIMULTANEOUS DRUG HIGH-CONTENT ANALYSIS SCREENING ON CELL-BASED MODEL FROM SEVERAL SALS PATIENTS

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Keywords: biomarkers, HCA, drug screening

Background: The causes for most cases of ALS (sporadic ALS (sALS)) are unknown, and the clinical course is highly variable, suggesting that multiple factors underlie the disease mechanism and therefore personalized ALS platforms for drug treatment need to be developed. To this end, we have isolated specific biomarkers found in non-neuronal samples of sALS patients and identified disease-related phenotypic signatures.

Results: We have recently found by Q-RTPCR analysis four novel ALS potential biomarkers in non-neural tissues from sporadic ALS patients in bone marrow mesenchymal stem cells (hMSC) and peripheral blood leukocytes that may have direct diagnostic and pathological implications in the disease (1, 2). Moreover, we have solid evidence that hMSC are ideal tools for image-based high-content analysis (HCA) screening assays aimed for drug high-throughput screening (HTS), since these cells can be kept at defined culture conditions without serum (3, 4).

In fact ALS-hMSCs show significant differences in various cell phenotypic signatures that are relevant to HCA assay development. The sALS cells respond differently to stress induction as compared to non-ALS controls. Recently we have discovered that these four potential ALS biomarkers are differentially expressed also in neuronal and non-neuronal tissues of the transgenic mouse ALS model SOD-1[(693A)] as compared with wild-type littermates at different times within the 120 days of disease progression (2). Importantly, these results support the physiopathological, diagnostic and prognostic relevance of the ALS biomarkers detected in hMSC and blood samples of sALS patients.

Discussion and conclusion: We propose a new strategy for personalized drug screening that will be based on ALS-associated biological cell phenotypes and molecular biomarkers expression in cell samples of ALS patients. We hope that from this screening, we will be able to identify personalized drugs or drug combinations for future treatment of the ALS patients involved in the study.

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P294   CHROMOSOMALLY MODIFIED MESENCHYMAL STEM CELLS SECRETING GDNF, IGF-1, AND HGF ATTENUATE DISEASE PROGRESSION IN AN ALS ANIMAL MODEL

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Keywords: trophic factor, stem cell, transplantation

Background: Stem cell transplantation and the injection of neuronal trophic factors are not necessarily mutually exclusive. We might therefore expect to obtain synergistic effects when combining both therapies for the treatment of a neuro-degenerative disorder such as amyotrophic lateral sclerosis (ALS). Using a human artificial chromosome (HAC) system, we previously established mesenchymal stem cells (MSCs) that simultaneously express glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF). Following transplantation of these cells into an ALS mice model, we also confirmed the efficacy of the transplantation on the ALS disease phenotypes.

The aims of the present experiments were to determine the best parameters for effective transplantation, such as when the transplantation should be conducted and how many cells should be administered for therapy.

Methods: The chromosomally modified MSCs were transplanted into 60-, 80-, 100-, or 120-day-old, high-copy SOD1[G93A] transgenic mice via the fourth ventricle, from which the cells distributed throughout the spinal cord. Littermate-, age- and sex-matched mice received sham operations or were transplanted with MSCs devoid of the chromosomal modification. The ALS mice model used in the experiments demonstrated ALS symptoms around 120 days and mice expired around 150 days of age. From 1 week prior to the transplantation until death occurred, body weight and the hind limb extension reflex score were measured once a week. FK-506, an immunosuppressive agent, was administered orally to prevent MSC rejection.

Results: In the mice transplanted at 80 and 120 days of age, there were no beneficial results in terms of the age of onset, death, or disease duration compared to control mice. In the mice transplanted at 60 days of age, there were encouraging results with delayed death and increased duration of disease in the treated mice compared to the controls. Where mice were transplanted at 100 days of age, there was statistical significance in terms of the age of death and disease duration, this effect was not seen in the mice transplanted with MSCs without chromosomal modification. When comparing the number of cells used for transplantation, transplantation with

2 million cells showed a greater benefit than that observed with 6 million cells.

Conclusions: The chromosomally modified MSCs delayed the onset of death and increased the duration of disease in the ALS models with mice treated at around 100 days of age, with 2 million cells demonstrating the best transplantation outcome. *In vivo* cell tracing, biochemical and histological investigations are now underway.

P295   NOVEL FEATURES OF ADULT RAT FACIAL MOTONEURONE RESCUE BY A MUSCLE-DERIVED ISOFORM OF IGF-1

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Background: We previously found that gene transfer of an isoform of insulin-like growth factor-1 (IGF-1) that is expressed by active muscle (termed Mechano-Growth Factor, MGF) rescued 80% of adult rat motoneurones following facial nerve avulsion when 80% would have died (1).

Objectives: In this study, we tested the possibilities that (i) the 24aa C-terminal MGF peptide (MGF24) delivered at the time of nerve injury is also neuroprotective; (ii) MGF24 acts via the IGF-1 receptor; (2) and (iii) MGF24 acts through activation of protein kinase C (3).

Methods: The right facial nerve was avulsed as it emerged from the stylomastoid foramen in groups of 5–6 anaesthetised adult (3m) Sprague-Dawley rats (4). In one group, 10μl of 1μg/μl MGF24 in saline was injected into the foramen immediately after avulsion. In two other groups, MGF24 was co-injected with 1μg/μl of either (i) an antibody to the rat IGF-1 receptor or (ii) the protein kinase C inhibitor GF109203X. Control groups received avulsion only or 10μl of either (i) saline, (ii) 1μg/μl liver-type IGF-1 or (iii) 1μg/μl glial-cell derived neurotrophic factor (GDNF). Anaesthetised rats were perfused with fixative 1 month later and numbers of motoneurones were determined stereologically (4).

Results: One month following avulsion only and avulsion plus saline, 80% of motoneurones were lost ipsilaterally. This loss was reduced to 50% by IGF-1 and MGF24 and 20% by GDNF (p < 0.05 vs. avulsion only). Co-injection of MGF24 with the IGF-1 receptor antibody or the Protein Kinase C inhibitor resulted in 53% and 60% loss of motoneurones, respectively (p < 0.05 vs. avulsion only).

Discussion and conclusion: Our results concur with several published reports that neuronal rescue by MGF is independent of the IGF-1 receptor, but they also indicate that adult motoneuronal rescue by MGF24 does not require protein kinase C activation. Whether this is a novel feature of MGF per se, or whether it is specific to our experimental model, remains to be determined.

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P296   THE BIOLOGICAL RELEVANCE OF CORTICOSPINAL MOTOR NEURONS TO MOTOR NEURON DISEASES

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Background: Corticospinal motor neurons (CSMNs) have a unique executive function in the brain. They receive, integrate, translate and transmit cerebral cortex inputs to spinal targets, acting as the spokes person for the cerebral cortex especially for motor neuron circuitry. Even though it is the pre-motor neurons that determine CSMN function, it is CSMN that connects the brain and the spinal cord for the execution and modulation of most of the voluntary movement in humans. Therefore, the health and connectivity of CSMN is centrally important for our movement, and CSMN degeneration leads to many motor neuron diseases contributing to paralysis in patients. Understanding mechanisms that underlie selective vulnerability and progressive degeneration of CSMN is critically important for building effective treatment strategies for motor neuron diseases, in which voluntary movement is impaired. Studying the details of CSMN biology has been limited due to the complexity of the cerebral cortex and the limited numbers of CSMN. However, recent developments allow their visualization, isolation and detailed investigation at different stages of the disease.

Results: We found that CSMN show varied transduction efficiency to different AAV serotypes, and that it can be retrogradely transduced by AAV2-2 in the most effective way. Most importantly, introduction of eGFP gene into constructs exposed the cytoarchitectural details of CSMN and revealed that apical dendrite degeneration is an early event in CSMN degeneration and lack of spines especially on apical dendrites suggest early cortical modulation defects in ALS.

Generation of a novel reporter line, the UCHL1-eGFP mice, which express eGFP under the UCHL1 promoter genetically labels CSMN and allows their detailed cellular analysis *in vivo*. Such studies uncovered autophagy as an intrinsic mechanism involved in CSMN apical dendrite degeneration, and begins to reveal the intrinsic mechanims that are involved for their vulnerability.

Discussion: Our ongoing studies shed light onto upper motor neurons as they reveal the intrinsic and extrinsic factors that are involved in CSMN vulnerability and progressive degeneration. We believe that further investigation into CSMN biology will ultimately help to develop effective

treatment strategies for ALS and related motor neuron diseases.

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P297   CHRONIC TREATMENT WITH LITHIUM DOES NOT IMPROVE NEUROMUSCULAR PHENOTYPE IN A MOUSE MODEL OF SEVERE SPINAL MUSCULAR ATROPHY

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Keywords: lithium, spinal muscular atrophy, SMNdelta7 mouse

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by defective levels of the survival motor neuron (SMN) protein. SMA causes spinal motoneuron (MN) loss and progressive muscle paralysis. There is no effective therapy for this disease. Although different strategies focused on increasing the expression of functional SMN protein have been assayed, numerous SMN-independent therapeutic approaches have been demonstrated to have potential effectiveness in improving SMA phenotype in mouse models and clinical trials (1). Recent works have shown that compounds which inhibit GSK-3β activity are effective in promoting MN survival and ameliorating lifespan in models of MN diseases including SMA (2).

Objectives: Taking into account the reported neuroprotective actions of lithium (Li) through the inhibition of GSK-3β in different studies (3), and the controversial effects found in ALS (4), we tested its potential efficiency as a therapeutic agent in a mouse model of SMA.

Methods: CD1 non-transgenic animals, and Smn$^{-/-}$; SMN2$^{+/+}$; SMNΔ7$^{+/+}$ (SMNΔ7) mice and their WT littermates were used in this study. Mice were daily treated with subcutaneous injections of different doses of LiCl starting on P1. Li plasma concentration was determined using atomic absorption spectrophotometry. Doses of LiCl were administered to achieve a plasma concentration of Li similar to the therapeutic range in humans (0.6–1.5 mEq/L). Motor behavioural tests, and histolopathological and western blot analysis in spinal cord and skeletal muscles were performed.

Results: Chronic treatment with Li, initiated before the appearance of disease symptoms, although inhibited GSK-3β, did not improve the median survival, motor behavior, and spinal MN loss linked to SMA. Li administration did not either ameliorate the microglial and astroglial reaction in the spinal cord or the depletion of glutamatergic synapses on MNs observed in SMNΔ7 animals. Moreover, Li treatment did not mitigate muscle atrophy or calcitonin gene-related peptide (CGRP) downregulation in the neuromuscular junctions linked to the disease (5). However, a significant reduction in apoptotic cell death found in the skeletal muscle of SMA mice (5) was observed after Li treatment.

Discussion and conclusion: These results demonstrate that despite its inhibitory action on GSK-3β, chronically administered Li has no beneficial effect on lifespan or neuromuscular dysfunction of SMN 7 mice.

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P298   GHRELIN ATTENUATES DISEASE PROGRESSION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ghrelin, SOD1^{G93A} mouse, food intake

Background: Ghrelin is a stomach-derived peptide hormone which is identified as an endogenous ligand for growth hormone secretagogue receptor 1a (GHS-R1a), and stimulates growth hormone (GH) secretion from the anterior pituitary gland. Ghrelin is also known to regulate energy homeostasis by stimulating food intake and promoting adiposity via a GH-independent mechanism. Recently, growing interest has centred on the role of energy metabolism in amyotrophic lateral sclerosis (ALS).

Objectives: The aim of the current study was to examine the effects of ghrelin in a mouse model of ALS (SOD1^{G93A} mice).

Methods: In study 1, Ad libitum fed SOD1^{G93A} mice were treated with Ghrelin (50 µg/day, n = 15) or vehicle (saline, n = 15) starting at 10 weeks of age. Food intake, body weight change, forelimb grip strength and survival period were analyzed. In study 2, Ad libitum fed SOD1^{G93A} mice were treated with Ghrelin (50 µg/day, n = 8) or vehicle (saline, n = 7) and wild-type (WT) mice were treated with vehicle (saline, n = 8), starting at 10 weeks of age. Gene expression levels in skeletal muscle and number of motor neurons in spinal cord were analyzed. In study 3, WT mice were treated with vehicle under ad libitum fed condition (n = 9) or food-restricted conditions (n = 8) and food-restricted SOD1^{G93A} mice were treated with Ghrelin (50 µg/ day, n = 15) or vehicle (saline, n = 13) starting at 10 weeks of age. Food-restricted mice were provided with approximately 90% of the mean amount of food consumed by ad libitum fed SOD1^{G93A} mice. All of the food-restricted mice consumed all food provided during the experiment. Body weight change, lean mass change, forelimb grip strength, gene expression levels in skeletal muscle and number of motor neurons in spinal cord were analyzed.

Results: Ghrelin treatment significantly extended survival period, increased food intake and body weight change,

suppressed forelimb grip strength reduction and prevented motor neuron loss in spinal cord compared with vehicle group in ad libitum fed condition. On the other hand, ghrelin treatment did not suppress the reduction of forelimb grip strength and did not prevent motor neuron loss in food restricted condition. Even in this condition, ghrelin treatment suppressed body weight and lean mass loss and suppressed gene expression which is related to muscle atrophy.

Conclusion: These results demonstrate for the first time that ghrelin significantly attenuates disease progression in SOD1[G93A] mice mainly through its orexigenic effect.

P299   INHIBITION OF EXTRACELLULAR CYCLOPHILIN A AS A POSSIBLE THERAPEUTIC TARGET FOR ALS

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Keywords: SOD1, cyclosporin A, inflammation

Background: Cyclophilin A (CypA) is an abundant and ubiquitously expressed multifunctional protein. Its best characterized property is the peptidyl-prolyl *cis-trans* isomerase activity. It is also secreted extracellularly where it has a proinflammatory cytokine-like behaviour. We found that CypA is a translational biomarker of ALS (1), is up-regulated during disease progression, is sequestered in insoluble aggregates (2) and is aberrantly secreted extracellularly (eCypA), as demonstrated by high levels of the protein in the cerebrospinal fluid in both ALS patients and SOD1[G93A] animal models. eCypA plays major roles in inflammatory mechanisms, and these functions are mediated by the interaction with its receptor, CD147. Preliminary data suggested that eCypA was specifically toxic for motor neurons. We hypothesized that astrocytes expressing mutant SOD1 secrete high levels of CypA that exerts a specific toxic effect on motor neurons. The recent discovery of a cyclosporine A (CsA) derivate (MM-218) able to selectively inhibit eCypA allowed us to develop a possible pharmacological approach based on modulation of eCypA function.

Objectives: The aim of this study is to test novel therapeutic approaches based on the modulation of the extracellular function of CypA, in both *in vitro* and *in vivo* models of ALS.

Methods: We used an *in vitro* model consisting of astrocyte-spinal neuron co-cultures, expressing or not SOD1[G93A]. In this system, we demonstrated that expression of mutant SOD1 in both cell populations causes selective and spontaneous loss of large motor neurons.

Results: We confirmed that conditioned media from astrocyte-spinal neuron co-cultures expressing SOD1[G93A] present high levels of eCypA. To study the specificity and mechanism of the toxic effect of CypA, we investigated CD147 expression

in the SOD1[G93A] mouse model of ALS. We observed increased expression of CD147 in motor neurons in the presence of the SOD1 mutation. We then tested the effect of MM-218 and four additional CsA analogs on the astrocyte-spinal neuron co-cultures expressing mutant SOD1. We observed that chronic treatments with eCypA inhibitors were not toxic for motor neurons and could rescue motor neurons in a specific range of concentrations.

Discussion and conclusion: The eCypA toxic activities on motor neurons are likely mediated by the interaction with CD147. SOD1[G93A] motor neurons *in vivo* are possibly more vulnerable to eCypA because of a higher expression of CD147. We demonstrated that inhibiting extracellular CypA with MM-218 rescued SOD1[G93A] motor neurons. Thus, we confirmed that modulating extracellular activity of eCypA is a potential novel pharmacological approach. Starting from these preliminary data, we are now developing a therapeutic approach in the SOD1[G93A] mouse model.

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P300   APO-H-FERRITIN INFUSION AS A THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: iron, SOD1 G93A, mouse

Background: Iron misregulation and deposition are consistent features in humans with amyotrophic lateral sclerosis (ALS) and in animal models of the disease. This aberrant iron homeostasis can induce oxidative stress and has been implicated in disease pathogenesis. A recent study by Jeong et al. (1) demonstrated that chelation of iron with a synthetic compound favourably impacts disease progression in the murine model of ALS. Therefore, it has become increasingly appreciated that appropriate management of iron is disrupted in ALS and restoring iron regulation may be an attractive therapeutic strategy.

Intracellular iron homeostasis is maintained by a number of proteins working in synergy; of particular note is H-ferritin. The ferroxidase activity of this protein limits the formation of reactive oxygen species, and both over-expression and exogenous application of ferritin have been shown to be neuroprotective. We propose that H-ferritin is advantageous over a chemical chelator to bind iron because this protein is a naturally occurring ionophore that can redistribute bound iron using pathways already present in the body, and the biological fate of the recycled iron is not detrimental.

Objectives: The objective of this work is to determine whether exogenous application of iron-poor H-ferritin (apo-H-ferritin) into the brain is a means to provide neuroprotection. Primary outcome measures are to evaluate whether infusion of apo-H-ferritin into the lateral ventricles delays disease onset and extends lifespan in the murine model of ALS.

Methods: At 70 days of age, mice with the SOD1^{G93A} mutation underwent surgery to implant a mini-osmotic pump and cannula to deliver continuous infusion of apo-H-ferritin into the lateral ventricle. Pumps were replaced 28 days after the initial surgery to maintain the H-ferritin levels. Onset of disease was behaviourally assessed by performance on the rotarod apparatus, and endpoint was determined by the inability of the animal to right itself within 30 seconds of being placed on its side.

Results: Mice that received infusion of apo-H-ferritin at a concentration of 2.0 mg/ml demonstrated a delay in disease onset and a modest extension of lifespan. Infusion of apo-H-ferrtitin at 4.0 mg/ml was of limited benefit but did not accelerate onset or endpoint as compared to the no surgery control group.

Discussion and conclusion: The data from these experiments suggest that iron chelation using the natural ionophore H-ferritin favorably impacts disease progression the SOD1^{G93A} mouse model of the disease. Our data indicate that further exploration and assessment of apo-H-ferritin as a novel strategy to treat ALS is warranted.

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P301   ADMINISTRATION OF ANTIBODIES FOR MISFOLDED SOD1 PROLONG SURVIVAL IN THE SOD1-G93A MOUSE

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Keywords: misfolded, SOD1, in vivo

Background: Since the identification of mutations in the superoxide dismutase 1 (SOD1) gene as a cause of amyotrophic lateral sclerosis (ALS), substantial efforts have been made to understand how mutations in SOD1 trigger motor neuron degeneration. Recent work has focused on the connection between toxicity and the propensity of mutant SOD1 protein to misfold. Mice expressing mutant SOD1 recapitulate many of the pathological and clinical features of ALS. Several reports have shown that targeting SOD1 by active or passive immunization can prolong survival.

Objective: We sought to rank a panel of antibodies obtained from AviTix and Amorfix to misfolded SOD1 in terms of their affinity and selectivity for mutant, denatured and oxidized SOD1. The top candidates were evaluated for their therapeutic potential in the SOD1-G93A mouse model.

Methods: Antibodies specific to misfolded SOD1 were profiled for their binding affinities for native or misfolded SOD1 and their abilities to immunoprecipitate mutant SOD1. One highly specific antibody was used to develop an ELISA for misfolded SOD1. Antibodies were chosen for *in vivo* assessment in the SOD1^{G93A} mice using chronic and acute dosing paradigms, where SOD1 levels were quantified in the spinal cord. Two survival studies (n = 14 and n = 20) were run in female B6 SOD1^{G93A} mice dosed weekly with 30 mg/kg intraperitoneally starting around day 50 until day 156. Mice received either a control antibody or one of 2 anti-misfolded SOD1 antibodies. Rotarod performance, body weight, onset and survival were measured. Drug levels in the spinal cord and blood plasma were also measured.

Results: Initial characterization led to the selection of two antibodies, B8H10 and 3H1, for survival studies in the B6 SOD1^{G93A} mouse model. Both antibodies delayed loss in body weight and increased survival when dosed chronically. An 8- to10-day improvement in median survival was observed. No changes in rotarod testing were observed, nor were any significant changes detected in native or misfolded SOD1.

Discussion: Antibodies with good selectivity for misfolded SOD1 were identified. Treatment of female B6 SOD1-G93A mice expressing mutant SOD1 with these antibodies improved survival, but did not reduce levels of misfolded SOD1 in the spinal cord or improve motor function as assessed by rotarod. This discrepancy warrants further investigation, such as assessment of dose-responsiveness of the survival effect and demonstration of central target engagement.

Conclusion: Treatment of SOD1^{G93A} mice with antibodies specific to misfolded SOD1 improves survival, but additional work to understand the mechanism of action is needed.

P302   NEUROPROTECTIVE AND IMMUNOMODULATORY EFFECTS OF THE SIGMA-1 RECEPTOR (S1R) AGONIST PRE-084, IN A MOUSE MODEL OF MOTOR NEURON DISEASE NOT LINKED TO SOD1 MUTATION

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Keywords: Sigma-1 receptor, neuroprotection, neuroinflammation

Background: *Sigma-1 receptors* (S1Rs) reside at the mitochondrion-associated ER membrane, where they exert

chaperone-like activity and act as inter-organelle modulators of Ca^{2+}-homeostasis, ER-stress and cell survival. S1R-gene mutations have been associated with familial forms of FTLD-MND (1) and of juvenile ALS (2). Knockout of S1R in SOD1^{G93A} mice accelerates disease progression (3), whereas treatment with PRE-084 improves locomotor function and motor neuron (MN) survival, supporting the hypothesis that S1R may represent a new therapeutic target for ALS (4).

Objectives: We aimed at validating S1R as therapeutic target for MND pathologies unrelated to SOD1 mutation such as sporadic ALS. The L967Q-Vps54 mutant Wobbler (wr) mouse was used as a model of MND not linked to SOD1 mutations (5,6).

Methods: Wr mice were treated with PRE-084 (0.25 mg/kg i.p.) from 4 to 12 weeks of age. Locomotor behaviour was assessed twice a week. Analyses of MN survival, astrocyte, oligodendrocyte and microglial (CD11b) markers, S1R protein levels and cellular distribution were performed at the end of treatment.

Results: In healthy mice, S1R was mainly present in spinal cord MN, co-localizing with ER-markers and was detectable in oligodendrocytes and myelin sheaths. In symptomatic mice, S1R increased in the surviving MN and it was detectable in some hypertrophic astrocytes and reactive microglia. PRE-084 significantly improved MN survival (26.5%) and motor performance after the week 4 of treatment. PRE-084 reduced astrogliosis and increased CD11b$^+$ cells without changing the average density of CD11b labelling. Evaluation of microglial phenotypes highlighted a significant increase in the number of CD68$^+$ cells in the white matter of PRE-084-treated mice. The majority of CD68$^+$ cells were also CD206$^+$ and localized close to radial glial cells.

Discussion: The increase in S1R in MN of symptomatic and PRE-084-treated wr mice, together with the beneficial effects exerted by PRE-084 on neuroprotection and motor symptoms, supports S1R as a molecular player crucially involved in the endogenous reaction to pro-degenerative stressors. Since behavioural effects are observed after 4 weeks from the beginning of treatment, sustained stimulation of S1R may be necessary to activate pathways associated with cellular plasticity. Microglia reactivity affects disease progression in ALS. Thus, a shift from an inflammatory M1 to a M2 phenotype (CD206$^+$) involved in tissue restoration may be part of the pro-regenerative response, induced by the S1R agonist.

Conclusions: Our results support pharmacological manipulation of S1R as a promising strategy to cure MND pathologies unrelated to SOD1 gene mutation. An immunomodulatory effect may be part of the mechanism of MN protection mediated by S1R.

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P303 CHANGES IN ENDOCANNABINOID RECEPTORS AND ENZYMES AND BENEFICIAL EFFECTS OF A SATIVEX®-LIKE COMBINATION OF PHYTOCANNABINOIDS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: endocannabinoid system, Sativex, neuroprotection

Background: Different cannabinoid compounds, ie Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabinol, selective CB_2 receptor agonists or fatty acid amide hydrolase (FAAH) inhibitors, afforded neuroprotection in the experimental model of amyotrophic lateral sclerosis (ALS) generated by overexpression of a mutated form of superoxide dismutase-1 (SOD-1) (1). By contrast, these mice have been poorly studied to determine the alterations caused by the disease in those elements of the endocannabinoid system targeted by the above treatments.

Objectives: In the present study, we addressed two objectives: (i) to analyze the changes in endocannabinoid receptors and enzymes in the spinal cord of SOD-1 transgenic mice at an advanced phase in the disease progression (17- to 18-weeks old); (ii) to evaluate the cannabis-based medicine SativexÒ, which is a combination of botanical extracts enriched in both Δ^9-THC and cannabidiol (CBD), as a disease-modifying therapy in this experimental ALS model, based on the potentiality of this combination to act through different mechanisms frequently activated by cannabinoid compounds.

Methods: i) To analyze the changes in endocannabinoid system components, we use RT-PCR analyses of untreated animals at several disease stages. ii) Pharmacological experiments consisted of a daily administration of Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts, at a dose of 40 mg/kg (equivalent to 20 mg/kg for each phytocannabinoid), starting when both wild-type and SOD-1 transgenic mice were 10 weeks old (the first symptoms in these animals typically appear at this age).

Results: Our biochemical studies proved a significant increase in CB_2 receptors and NAPE enzyme in SOD-1 transgenic males, and only CB_2 receptors in SOD-1 transgenic females. Moreover, trends toward an increase were also found for MAGL and DAGL enzymes in both genders, but not in CB_1 receptors and FAAH enzyme.

Our results demonstrated that the treatment of SOD-1 transgenic mice with the Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts: (i) partially attenuated the weight loss typical of these animals, but this positive effect was only found in males not in females; (ii) delayed the progression of neurological deficits, in particular in females; and (iii) slightly increased animal survival, an effect observed in both genders.

Discussion and conclusion: In summary, our results provide support to the possibility that Sativex® may serve as a novel disease-modifying therapy in ALS, a disorder with a poor therapeutic outcome at present with only one medicine already approved, Rilutek®, but with a modest efficacy on disease progression. More preclinical studies in additional models of ALS, that is, TDP-43 transgenic mice, will be necessary before clinical evaluation of SativexÒ in ALS patients.

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P304 EFFECTS OF DEXPRAMIPEXOLE ON WHITE BLOOD CELLS IN A MINI-PIG TOXICOLOGY STUDY AND FROM TWO CLINICAL TRIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: eosinophilic-associated diseases, dexpramipexole, hematology

Background: Dexpramipexole is a small, orally bioavailable molecule with demonstrated cytoprotective properties in *in vitro* and *in vivo* preclinical studies. Dexpramipexole is in development for the treatment of ALS. The potential for dexpramipexole in the treatment of eosinophilic-associated diseases (EAD) became apparent from a 39-week toxicology study in mini-pigs, and from Phase 2 and Phase 3 clinical trial results.

Objectives: To determine whether treatment with dexpramipexole in ALS subjects resulted in levels of granulocyte reduction similar to that noted in pre-clinical toxicology studies.

Methods: The Phase II, 2-part study randomized 102 subjects and the phase III study randomized 943 subjects in a double-blind, placebo-controlled trial to assess the safety and efficacy of dexpramipexole in ALS, respectively. Subjects were randomized to 25mg, 75mg, or 150mg dexpramipexole twice daily, or placebo for up to 9 months (Phase II), or 150mg dexpramipexole twice daily, or placebo for up to 18 months (Phase III). Monthly CBCs were obtained in both studies. The chronic minipig study was performed to evaluate the potential toxicity of dexpramipexole after administration for up to 39 weeks. Three treatment groups of Gottingen Mini-pigs® were administered dexpramipexole, at respective dose levels of 7.5, 25, and 75/50 mg/kg/day.

Results: In a chronic toxicology study in Gottingen minipigs, a time- and dose-dependent decrease in eosinophils was observed. In CL201, part 1 of this study, eosinophils were reduced at 12 weeks in the 150- and 300-mg groups. In part 2 of this study, the 4-week washout showed a partial return to baseline. Patients were then re-randomized to 50 or 300 mg/day for 28 weeks, and the 300-mg group demonstrated a clear reduction in eosinophils. In the phase 3 EMPOWER trial, 82% of dexpramipexole-treated ALS patients experienced a 50% or greater decrease in blood eosinophil counts compared to baseline values with additional small reductions in neutrophils, basophils, monocytes, and lymphocytes. However, there were no clinically significant changes in monocytes and lymphocytes. Neutropenia (ANC, $< 1.5 \times 109/L$) was observed in 29 dexpramipexole-treated patients (6.1%) and 8 (1.7%) patients receiving placebo, and was reversible upon withdrawal of treatment.

Discussion and conclusion: These empirical observations in essentially three human studies and a large animal toxicology study demonstrate a very robust effect of dexpramipexole on blood eosinophil counts at doses that appear to be well tolerated. The slowly developing and sustained reduction in eosinophils, as well as other cells arising from a multipotential hemapoietic stem cell, suggests that dexpramipexole might diminish hematopoiesis, accelerate apoptosis, or promote migration of eosinophils into tissue, or perhaps a combination of several of these effects. As dexpramipexole is well tolerated in humans following exposures up to 18 months, it may represent a novel therapeutic approach for the treatment of EAD.

P305 DETERMINING THE SAFETY OF L-SERINE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) AT VARIED DOSES

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Keywords: BMAA, L-Serine, environmental causes

Background: Previous studies into the Guamian ALS–Parkinson's dementia complex have identified β-methylamino-L-alanine (BMAA), as the potential neurotoxin responsible for ALS. BMAA is a non-essential amino acid produced by cyanobacteria. The hypothesis has been that some individuals are vulnerable to BMAA deposition into their central nervous system where it is incorporated into proteins which can then serve as a reservoir for this neurotoxin. Recent evidence suggests that BMAA may be mis-incorporated in the place of the amino acid serine in brain proteins. It has been demonstrated in mammalian neuronal cell cultures that exogenous L-serine could prevent the BMAA neurotoxin from being mis-incorporated into proteins, thereby preventing cell death. Other studies have demonstrated that very high doses of L-serine may compete with the transport of a number of non-essential amino acids across the blood–brain barrier via the y^+ transporter. These findings lead us to believe that high doses of L-serine could possibly stop the mis-incorporation of BMAA into brain proteins which in turn would slow or even abate the progression of ALS.

Objective: To determine the safety and tolerability of L-serine given at 0.5 g twice daily (BID), 2.5g BID, 7.5g BID

or 15g BID for six months in patients with sporadic ALS, and to measure levels of BMAA in CSF, blood and urine pre- and post treatment.

Methods: Twenty patients diagnosed with sporadic ALS on stable doses of riluzole were enrolled. Eligible patients were randomized in a double-blinded fashion to four separate doses for 6 months. CSF, blood and urine were collected and shipped to the Institute for Ethnomedicine for BMAA analysis at baseline and at month 6. ALS-FRS-R and safety laboratories were measured monthly. FVC and the quality of life visual analogue scale (QOLVAS) were measured at baseline and at months 3 and 6.

Results: To date, seven patients have been randomized and 4 patients have completed 3 months of therapy. The average of participants is 64-years of age, and average time of diagnosis to screening is 11 months. No drug-related side effects have been reported from any of the subjects. The rate of ALS progression as measured by monthly change in ALSFRS-R scores in the four patients who have completed 3 months of therapy is 0.5.

Conclusion: L-serine at different doses seem to be well tolerated in patients with ALS

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P306   INHIBITION OF NOX 2 NADPH OXIDASES AS A POTENTIAL TREATMENT FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: NADPH oxidase, neuroinflammation, ROS

Background: Neuroinflammation and oxidative stress are common features of multiple neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS). Chronic activation of microglia and production of proinflammatory and cytotoxic factors including reactive oxygen species (ROS) contribute to progressive degeneration of neurons. In a transgenic model of ALS, NADPH oxidase 2 (NOX2) expression is strongly increased and NOX2 represents a major source of ROS generation during the progression of the disease. Thus inhibition of NOX2 may represent a new promising strategy for the treatment of neurodegenerative disorders. However, no specific and potent NOX2 inhibitor is currently available.

Objectives: To identify novel NOX inhibitors; to evaluate the effect of NOX inhibitors on ROS production in an *in vitro* model of neuroinflammation; to develop new readouts to evaluate oxidative stress *in vivo*; and to evaluate the effect of NOX inhibitors on survival, motor function and oxidative stress in an *in vivo* model of ALS.

Methods: In order to identify small molecule NOX2 inhibitors, we performed a screen of a NINDS library using PMA-activated neutrophils and luminol-enhanced luminescence as a read-out. We tested the effect of selected compounds on ROS production in mouse microglial RA2 cells stably expressing SOD1^{G93A} and activated with LPS. We used LC/MS to quantify formation of superoxide anion in spinal cord of SOD1 G93A mice. We performed *in vivo* tests of selected compounds in SOD1^{G93A} mouse model of ALS.

Results: In search of NOX inhibitors, we identified a group of compounds of the phenothiazine family. Based on their potency of NOX2 inhibition, we selected the following compounds: prochlorperazine (IC50 = 2.4 ± 0.4 μM), promazine (IC50 = 7.8 ± 2.2 μM), perphenazine (IC50 = 3.9 ± 0.7 μM), thioridazine (IC50 = 2.2 ± 0.2 μM). We showed that thioridazine and perphenazine decrease production of O_2- and H_2O_2 in RA2 SOD1^{G93A} cells activated with LPS and this decrease is concentration dependent. We found that O_2- production is increased in the spinal cords of SOD1 ^{G93A} mice as compared to WT littermates. *In vivo* experiments suggest that thioridazine has a modest protective effect in SOD1^{G93A} mice by increasing their survival by 7 days (p = 0.028).

Discussion and conclusion: In conclusion, our preliminary results demonstrate that inhibition of NOX2 is a promising strategy for the treatment of neuroinflammation in neurodegenerative disorders such as ALS. Experiments evaluating the effect of these compounds on ROS production and oxidative stress *in vivo* are ongoing.

P307   CLINICAL TRIAL OF EDARAVONE IN AMYOTROPHIC LATERAL SCLEROSIS/ PARKINSONISM–DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Keywords: Kii ALS/PDC, Clinical trial, Edaravone

Background: Amyotrophic lateral sclerosis/parkinsonism–dementia complex of the Kii peninsula of Japan (Kii ALS/PDC) is a unique ALS with fronto-temporal dementia. The purpose of this study is to reveal clinical effects of Edaravone, a free radical scavenger, on the patients with Kii ALS/PDC.

Objective: Five patients with Kii ALS/PDC (ratio of men to women, 4:1; average age, 68.4 years; average duration of the illness, 9.4 years) were submitted for the study.

Method: Edaravone (30 mg per one time) was administered twice a week, as an intravenous drip infusion to the patients

with Kii ALS/PDC for 16 weeks. Vitamin C (2000 mg/day) and vitamin E (300 mg/day) were used concomitantly. The effect of Edaravone was evaluated using mini mental state examination (MMSE), ALS functional rating scale revised (ALSFRS-R), unified Parkinson's disease rating scale (UPDRS), apathy scale, frontal assessment battery (FAB) and clinical assessment for spontaneity (CAS). This study was approved with the ethical committee of Nansei town hospital.

Result: Two out of the five patients, who had relatively mild symptoms, showed improvement of spontaneity, and mental and physical activity. The mildest patient showed marked improvement in UPDRS, apathy scale and CAS. The other one showed no apparent change. The residual two patients, who were bed-ridden, showed irritability, violence and sexual deviation: Edaravone was given up for 2 months.

Discussion: On the presupposition that oxidative stress plays an important role on the pathomechanism of Kii ALS/PDC, we performed a clinical trial of the free radical scavenger, Edaravone, in five patients with Kii ALS/PDC. Edaravone may be effective in patients at an early stage of the disease. A further large-scale trial will be needed.

Conclusion: Edaravone was effective to Kii ALS/PDC in the early stages of disease.

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P308   SAFETY OF CENTRAL VENOUS CATHETER IN SELF-ADMINISTRATION OF CEFTRIAXONE IN ALS

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Background: ALS is a fatal motor neuron disease with only one disease-modifying FDA-approved treatment. There is a dire medical need for new disease modifying treatments. The clinical trial of ceftriaxone was initiated to address this need (1). Ceftriaxone requires twice daily intravenous administration; for this trial central venous catheter (CVC) and home administration by patient and/or caregiver were used, posing a novel challenge in neurological clinical trials. CVCs are associated with risk of infection especially in debilitated patients and have typically required licensed personnel to manage. Trial participants underwent standardized teaching and regular re-evaluation of subjects and caregivers on CVC line care.

Objectives: To compare the incidence of CVC infection in people enrolled in a trial of ceftriaxone with those reported in a retrospective analysis of Strategic Healthcare Program (SHP) database of home infusions using intravenous catheters (2).

Methods: The study of ceftriaxone for ALS is a multicenter, adaptive design clinical trial of ceftriaxone for ALS involving a total of 513 subjects (66 in Stage 2 and 447 in Stage 3) (1). Incidence of CVC-associated infection was collected from the study database for both Stages 2 and 3. Comparison data were obtained from SHP.

Results: In the 513 subjects in the ceftriaxone study, the incidence of CVC infection included line infection (0.23 per 1000 catheter days), exit site infection (0.12 per 1000 catheter days), for an overall CVC infection rate of 0.35 per 1000 catheter days. Overall SHP infection rates reported for tunnelled catheters were 0.70 per 1000 catheter days.

Discussion and conclusion: We found a lower incidence of CVC-associated infections in our study population compared to the rates reported by SHP. The intensive standardized teaching and regular re-evaluation of subjects and caregivers knowledge of CVC safety provided by licensed personnel, and the prohibition of blood draws from CVCs in the ceftriaxone trial are likely contributors.

CVC infection rates from the trial of ceftriaxone for ALS suggest that with specialized training, patients and caregivers can self-administer long-term IV medications via Hickman catheter safely. This opens the possibility for testing other IV drugs for neurodegenerative diseases such as ALS.

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P309   HEAT SHOCK FACTOR-1 (HSF-1) CONTROLS PATHOLOGICAL LESION DISTRIBUTION OF POLYGLUTAMINE-INDUCED MOTOR NEURON DISEASE

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Background: Adult-onset motor neuron diseases including amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA) share a common feature that disease-causing proteins selectively accumulate in specific regions despite a broad expression. Heat shock factor-1 (Hsf-1) regulates the expression level of Hsps, such as Hsp70, Hsp105 and Hsp40, which are molecular chaperones that play protective roles in the neurodegenerative process by refolding and solubilizing pathogenic proteins. To elucidate the role of Hsf-1 in the pathological lesion selectivity of motor neuron diseases, here we investigate the effect of this molecule on the pathogenesis of the SBMA mouse model. SBMA is a late-onset motor neuron disease caused by the expansion of a CAG repeats in the gene-coding androgen receptor (AR). This

disease affects susceptible regions, such as spinal anterior horn, brainstem, and pancreas, whereas the causative protein is ubiquitously expressed.

Methods: We performed immunohistochemistry and Western blotting of various tissues from wild-type; AR-97Q (SBMA model mouse: 97Q $^{+/-}$, Hsf-1 $^{+/+}$); and AR-97Q *Hsf-1+$^{/-}$* (heterozygous Hsf-1 knockout SBMA mouse: 97Q $^{+/-}$, Hsf-1 $^{+/-}$) mice using anti-Hsf-1, anti-Hsp70 and 1C2 antibodies. Moreover, we analyzed the effect of lentiviral over-expression of HSF-1 in this mouse model.

Results: Hsf-1 expression levels are associated with the accumulation of pathogenic AR in each region of SBMA mouse. For example, in the cerebellum of AR-97Q mice, there was a scarce accumulation of pathogenic AR in Purkinje cells, where Hsf-1 was expressed at a high level. Conversely, there were abundant polyglutamine-positive cells in the cerebellar granular cell layer, which showed poor immunoreactivity for Hsf-1. In heterozygous Hsf-1 knockout SBMA mice, abnormal AR accumulates in the cerebral visual cortex, liver, and pituitary, which are not affected in their genetically unmodified counterparts. In the spinal anterior horn and other parts of central nervous system of AR-97Q *Hsf-1* $^{+/-}$ mice, the accumulation of mutant AR was substantially increased through Hsp70 down-regulation.

Furthermore, the frequency of pathogenic AR accumulation around the lentiviral vector-injected area of the motor cortex and striatum where HSF-1 was highly expressed was decreased in comparison with that in the contralateral side without treatment. In addition, the neuron sizes of the motor cortex and striatum were significantly increased by the HSF-1 injection.

Discussion: These findings suggest that Hsf-1 contributes to the determination of the pathological lesion selectivity in SBMA.

P310   PROTEIN DISULPHIDE ISOMERASE IS PROTECTIVE AGAINST MUTANT SOD1, TDP-43 AND FUS PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: PDI, SOD1, ER stress

Background: Superoxide dismutase (SOD1), fused in Sarcoma (FUS) and Tar-DNA-binding protein-43 (TDP-43) are key proteins linked to amyotrophic lateral sclerosis (ALS) pathology. Whilst neurodegenerative mechanisms are not fully defined in ALS, dysfunction to the endoplasmic reticulum (ER) is increasingly implicated in the pathology. Protein disulphide isomerase (PDI) is a chaperone which also functions as disulphide isomerase in the formation and reduction of protein disulphide bonds. It is primarily located in the ER, but it is also found in other cellular locations. Our laboratory previously showed that over-expression of PDI is protective against

mutant SOD1. PDI has also been shown to co-localise with FUS and TDP-43-positive inclusions in ALS patients.

Objectives: Here we examined whether over-expression of PDI is also protective against FUS and TDP-43 cellular pathology, ER stress and mis-translocation in the cytoplasm. Furthermore, we examined the mechanism by which PDI is protective. A small molecule mimic of the PDI active site was examined *in-vivo* in SOD1 mice.

Methods: Wild-type and mutant TDP-43 and FUS constructs were co-transfected with PDI in neuronal cell lines; cellular pathological hallmarks, including translocation to the cytoplasm, ER stress and ER-Golgi trafficking were examined using confocal microscopy, immunocytochemistry and immunoprecipitation. Immunohistochemistry was performed using ALS patient tissues.

Results: Over-expression of PDI was protective in neuronal cell lines expressing either mutant TDP-43 or FUS against (i) translocation from nucleus to the cytoplasm; (ii) ER stress ;(iii) inhibition of ER-Golgi transport. Mutations of key residues in the PDI active site demonstrated that the disulphide interchange activity rather than the chaperone activity was necessary for its protective ability. Furthermore, we demonstrated that PDI in the cytoplasm, rather than the ER, is responsible for the neuroprotective activity. Moreover, the small molecular mimic of PDI which mimics its disulphide interchange activity rescued the loss of motor neurons and reduced ER stress in SOD1^{G93A} mice. PDI also co-localised with C9orf72-positive inclusions in ALS patient's motor neurons consistent with the notion that it has broad protective activity against multiple misfolded proteins in ALS

Conclusion: PDI is protective against the major misfolded proteins linked to ALS. The molecular mimic may therefore be a novel therapeutic target in multiple forms of ALS.

P311   POSSIBLE MITOCHONDRIAL TARGET ENGAGEMENT IN AN OPEN-LABEL TRIAL OF RASAGILINE FOR ALS

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Keywords: pharmacology, biomarkers, therapy

Background: Oxidative stress, mitochondrial dysfunction and apoptosis have been proposed as the cause of motor neu-

ron death in ALS. Rasagiline is FDA-approved for the symptomatic treatment of Parkinson's disease and has demonstrated neuroprotective activities against neurotoxins in neuronal cell cultures and in the SOD mouse model of ALS. *In vitro* experiments indicate that rasagiline stabilizes mitochondria under stress.

Objectives: The specific aim of this open-label screening study is to determine whether rasagiline is safe in this patient population. The secondary aims are to determine whether mitochondrial function is affected by rasagiline by comparing mitochondrial biomarker levels before and after drug treatment, and to obtain preliminary data on disease progression using the ALSFRS-R.

Method: This is a phase II multi-center open-label study in El Escorial probable or definite ALS who met our criteria. Subjects were treated with rasagiline 2 mg daily for 12 months. Biomarkers were obtained before treatment and at 6 and 12 months and included lymphocyte mitochondrial membrane potentials (two methods, JC-1 and Mitotracker); oxygen radical antioxidant capacity (ORAC) assay to measure antioxidative stress; BCL-2/BAX protein ratios using Western blot; and lymphocyte Annexin levels for apoptosis.

Results: Thirty-six patients enrolled at nine centers of the Western ALS study group and 23 patients completed 12 months of treatment. ALSFRS-R declined at a rate of 1.19 per month and was not significantly different from historical controls. Biomarker assays showed increased mitochondrial hyperpolarization (JC-1, fluorescence ratio at baseline = 0.54, 6 months = 0.62, 12 months = 2.43; p = 0.05; Mitotracker, percent fluorescence at baseline = 18.05%, 6 months = 28.18%, 12 months = 67.67%; $p < 0.05$). Annexin showed a decrease in cell apoptosis (percent fluorescence at baseline = 29.4%, 6 months = 27.95%, 12 months = 22.72%; $p < 0.05$), Bcl/Bax ratio showed anti-apoptotic cell conditions (baseline = 0.03, 6 months = 0.05, 12 months = 0.27; $p < 0.05$), ORAC showed antioxidant protection against oxidative stress (baseline = 4834.92μmol, 6 months = 6575.45μmol, 12 months = 5700.19μmol; $p < 0.05$).

Conclusion: We may have evidence of mitochondrial target engagement. Other explanations for the biomarker data also include: disease progression that may have changed the assay measurements, or technical components (equipment, experience, processing of samples). Inclusion of a placebo-treated group could help resolve this or, alternatively, re-measuring biomarker parameters after a drug wash-out period. This drug needs to be studied further in ALS and a Phase II placebo-controlled trial is underway.

P312 REGULATION OF IP3-RECEPTOR-MEDIATED CALCIUM SIGNALING AND CELL DEATH BY THE BH4 DOMAIN OF BCL-XL IN ALS ASTROCYTES

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Keywords: astrocytes, cell death, cell-penetrating peptides

Background: A major constraint to the comprehension of ALS pathogenesis has been long represented by the assumption that this disorder selectively affects motor neurons in a cell-autonomous manner. Yet, the increased knowledge of the complex cellular interactions that exist within the CNS has recently moved the focus of the investigations towards non-neuronal cells, particularly astrocytes. Astrocytes can damage motor neurons by secreting toxic factors, but they can play a deleterious role also by losing functions that are neurosupportive. Recently, we reported that a subpopulation of spinal cord astrocytes degenerates in the microenvironment of motor neurons in the hSOD1^{G93A} mouse model of ALS. Mechanistic studies *in vitro* identified a role for the transmitter glutamate in the gliodegenerative process via the activation of its IP$_3$-generating metabotropic receptor 5 (mGluR5) (1).

Objectives: The aims of the present project are threefold: i) to study the mechanism(s) underlying astrocyte degeneration in ALS, downstream mGluR5; ii) to investigate the impact of a biologically active peptide, consisting of the BH4 domain of Bcl-X$_L$ fused to the protein transduction domain of the HIV TAT protein (TAT-BH4), towards mGluR5-driven calcium (Ca^{2+}) signaling; and iii) to explore the therapeutic potential of TAT-BH4 *in vivo*, in ALS mice.

Methods: *Cell Cultures:* Primary astroglial cultures were prepared from spinal cord of newborn mice, as previously described (1, 2). *Ca^{2+} imaging:* astrocytes were plated on glass coverslips and loaded with Fluo4-AM. *In vivo* treatment: hSOD1^{G93A} mice were administrated daily 5 mg/kg TAT-BH4 peptide, intraperitoneally, starting at the age of 40 days.

Results: Much evidence indicates that non-physiological formation of IP$_3$ can prompt IP$_3$ receptor (IP$_3$R)-mediated Ca^{2+} release from the intracellular stores and trigger various forms of cell death. Based on this, here we investigated the intracellular Ca^{2+} signaling that occurs downstream of mGluR5 in hSOD1^{G93A}-expressing astrocytes. Contrary to wild-type cells, we found that stimulation of mGluR5 causes aberrant and persistent elevations of intracellular $^{Ca2+}$ concentrations in the absence of spontaneous oscillations. The interaction of IP$_3$Rs with the anti-apoptotic protein Bcl-X$_L$ was previously described to prevent cell death by modulating intracellular Ca^{2+} signals. In mutant SOD1-expressing astrocytes, we found that the sole BH4 domain of Bcl-X$_L$, fused to TAT, is sufficient to restore sustained Ca^{2+} oscillations and cell death resistance. Furthermore, chronic treatment of hSOD1^{G93A} mice with the TAT-BH4 peptide reduces astrocyte degeneration, slightly delays disease onset, and improves both motor performance and survival (2).

Discussion and conclusion: Our results highlight the glioprotective potential of TAT-BH4 and indicate this peptide as a novel therapeutic for the treatment of ALS.

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P313 DELETING EPHRIN-B2 FROM REACTIVE ASTROCYTES IS BENEFICIAL IN ALS

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Keywords: ephrinb2, astrocytes, rodents

Background: Recently it has been shown that the EphA4 receptor is a modifier of ALS. Genetic and pharmacological inhibition of EphA4 rescues the phenotype in the zebrafish model of ALS and increases survival in ALS rodent models. In ALS patients, an inverse correlation was found between EphA4 expression and disease onset. However, what the mechanism of action is has not yet been fully elucidated. Remarkably it is known that EphA4 interacts with both ephrin-a and ephrin-b ligands, which are also bound to the cell membrane by a GPI-anchor or a transmembrane domain, respectively. Several of the EphA4 interaction partners have been shown to be expressed on reactive astrocytes, microglia and oligodendrocytes. These cells play an important role in the pathogenesis of ALS and surround motor neurons which abundantly express EphA4.

A promising candidate for the Eph 4 bind partner is ephrin-b2 as this has been shown to be highly expressed by reactive astrocytes after spinal cord injury. In the spinal cord of WT SOD1 mice, ephrin-b2 was highly expressed in motor neurons while only faint expression could be detected in astrocytes. At symptomatic stages, the expression pattern changes and high immunoreactivity could be detected in astrocytes while the neuronal expression diminished. Similar results were obtained in spinal cords from ALS patients and controls. Although the expression pattern of ephrin-b2 changes during disease progression, the overall expression stays the same as checked by RT-PCR analysis.

Results: We hypothesised that deleting ephrin-b2 from reactive astrocytes might have a beneficial effect on ALS. For this purpose we crossed the conditional ephrin-b2 knockout mouse with a GFAP-specific Cre-line and the SOD1[G93A] ALS model. Even though the GFAP-Cre promoter shows leaky expression, we find delayed disease onset and prolonged disease duration. These results suggest that ephrin-b2 might play a role in modifying amyotrophic lateral sclerosis, but it will need further investigation.

P314 INTERLEUKIN-1 RECEPTOR ANTAGONIST TREATMENT OF ALS PATIENTS WITH PREDOMINANT LOWER MOTOR NEURON INVOLVEMENT

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Keywords: Anakinra, IL-1 receptor antagonist, neuroinflammation

Background: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease of adult onset. Neuroinflammation contributes to ALS disease progression. The familial ALS-linked mutant superoxide dismutase 1 (SOD1) activates the inflammasome and the secretion of IL-1b in microglia. Inflammasome-deficiencies or treatment with recombinant IL-1 receptor antagonist (IL-1RA, Anakinra) extended the lifespan of G93A-SOD1 transgenic mice and attenuated the inflammatory pathology (1).

Objective: The aim of this open-label phase-IIb-study was to evaluate the safety and tolerability of Anakinra in 20 ALS patients with a predominant presentation of lower motor neuron dysfunction PMA.

Methods: In an open-label phase-IIb-study (NCT01277315), we included 20 ALS patients (18 male, 2 female; ALS-FRSr = 40.7/48) with either a predominant (N = 9) or pure lower motor neuron degeneration (progressive muscular atrophy; PMA; n = 11). The patients were treated daily for 52 weeks with subcutaneous Anakinra (100 mg/day) in combination with oral riluzol. Serum levels of IL-1RA, IL-6 and IL-1ß were analysed, and the expression levels of the most common ALS-genes (SOD1 und C9orf72) were determined.

Results: As expected, there were very frequent skin reactions (58%) at the beginning of the therapy, which decreased in intensity and frequency during the treatment. Light to moderate headache was reported at least once by less than half of the patients (47%). We did not observe any serious side effects, but three patients dropped out during the trial (two due to ALS-related hypoventilation syndrome and one withdrew from the trial). Between 6 and 12 months of treatment, we found a statistically non-significant reduction of the intraindividual progression rate in patients with PMA compared to those with signs of both upper and lower motor neuron degeneration.

Discussion: Anakinra was well tolerated by ALS patients. In a subgroup of ALS patients presenting with PMA, there was a trend towards slower disease progression. This exploratory trial is limited in proof of efficacy by the small number of patients. Based on the safety and tolerability results of this study, a phase-III-study is justified.

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P315   IS IVIG TREATMENT WARRANTED IN PATIENTS WITH PROGRESSIVE ASYMMETRIC LOWER MOTOR NEURON LIMB WEAKNESS WITHOUT CONDUCTION BLOCK? A PROSPECTIVE, COHORT STUDY

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Background: Treatable progressive lower motor neuron syndromes (PLMNS), such as multifocal motor neuropathy (MMN), remain difficult to distinguish from lower motor neuron (LMN) predominant amyotrophic lateral sclerosis (ALS) early in the course of disease. Small case studies demonstrating successful treatment of selected patients, with asymmetric limb weakness that does not meet the diagnostic criteria of MMN, with conduction block (CB) have prompted clinicians to recommend a trial of intravenous immunoglobulin (IVIg) for patients with progressive and asymmetric distal LMN limb weakness without electrodiagnostic features of MMN. However, this treatment approach produces a significant burden on health care resources.

Objectives: To prospectively evaluate the likelihood of response to IVIg by patients presenting with progressive, asymmetric, pure LMN limb weakness, and to determine the clinical phenotype of those who respond.

Methods: The study prospectively recruited 31 consecutive patients with progressive, focal-onset LMN limb weakness, without evidence of clinical upper motor neuron signs, sensory, respiratory or bulbar involvement, or evidence of motor nerve conduction block on electrodiagnostic studies. Each patient underwent treatment with IVIg (2g/kg) for a minimum of 3 months. Electrodiagnostic studies, a neuromuscular symptom score and expanded Medical Research Council sum score were documented before and after IVIg treatment. The final diagnosis was determined after prolonged clinical follow-up.

Results: Only 3 out of 31 patients (10%) responded to IVIg. Of the remaining 28 patients, 43% developed UMN signs and were diagnosed with ALS, 32% developed bulbar and respiratory symptoms and were diagnosed with progressive muscular atrophy (PMA) and 25% developed progressive spreading LMN limb weakness and were diagnosed with probable PMA. All responders demonstrated distal upper limb (UL) onset weakness, EMG abnormalities confined to the clinically weak muscles, and a normal CK. This set of features was also identified in 31% of non-responders presenting with distal UL weakness. Gender, age at onset, number of involved limb regions and the duration of symptoms prior to treatment were not significantly different between groups. Significant side effects from IVIg therapy were reported by 39% of patients.

Discussion: The rate of response to IVIg in this series was considerably lower than previously published studies (response rates, 40–74%), possibly due to previous studies reporting selected groups of patients, and including patients with some electrophysiological features of demyelination, hence increasing the likelihood of an underlying inflammatory etiology. If IVIg treatment was limited to patients in this series who demonstrated the clinical and laboratory features found in the responders, a rate of response of 50% would have been achieved.

Conclusion: The findings of the present study do not support uniform use of IVIg in patients presenting with progressive, asymmetric LMN limb weakness without conduction block, but rather appropriate patient selection based on clinical and laboratory findings.

P316   2B3-201, GLUTATHIONE PEGYLATED LIPOSOMAL METHYLPREDNISOLONE, ENHANCES BRAIN DELIVERY OF METHYLPREDNISOLONE AND REDUCES PATHOLOGY IN A MOUSE MODEL OF ALS

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Background: Study of ALS pathophysiology has revealed neuro-inflammation to be a prominent feature across a range of research techniques. Both in ALS patients and in transgenic mice, over-expressing mutant superoxide dismutase-1 ($SOD1^{G93A}$), activation or proliferation of microglia and astrocytes have been observed (1). The anti-inflammatory glutathione pegylated liposomal methylprednisolone (2B3-201) demonstrated superior efficacy and reduced side effects compared to free methylprednisolone (MP) in rodent models of neuro-inflammation (2).

Objective: To investigate the pharmacokinetics, brain delivery, and safety of 2B3-201, and its efficacy in reducing the neuro-inflammatory pathology associated with the transgenic *SOD1* mouse model of ALS.

Methods: 2B3-201 was investigated in a pharmacokinetic and biodistribution studies in both rats and in *SOD1* mice and compared to free MP. In addition, CNS behavioural and repeat-dose toxicology studies were carried out in rats at several dose levels. Next, the efficacy of 2B3-201 was investigated in *SOD1* mice, in which 60-day old animals received 8 weekly intravenous injections with either 2B3-201 or free MP (both at 10 mg/kg). *SOD1* and wild-type (WT) animals both receiving saline were used as controls. Efficacy measurements included motor function (rotor-rod), and at the endpoint (116-day-old mice), T2-weighted MRI was used to detect signal intensity in brainstem nuclei (V, VII and XII) and correlated with immunohistochemistry for astrocytes (GFAP) and microglia (Iba1).

Results: 2B3-201 showed an enhanced plasma circulation in rats (half-life of approximately 7 h versus several minutes for free MP), and higher sustained levels of 2B3-201 in brain and spinal cord of *SOD1* mice. 2B3-201 did not lead to the psychotic-like behavioural effects observed in rats with free MP treatment. Repeated weekly administrations of 2B3-201

were well tolerated in rats, while the same weekly doses of free MP caused side effects, such as urine retention. Compared to WT mice, all *SOD1* groups showed a significant decrease in motor performance from 100 d, without any significant treatment effects being observed. All *SOD1* mice showed a significant increase in signal intensity on T2-weighted MR images compared to WT mice ($p < 0.001$), which may reflect the combination of neuronal vacuolation and glial activation in these motor nuclei. Treatment with 2B3-201 reduced T2 hyperintensity, to a greater extent than free MP ($p < 0.01$).

Discussion and conclusion: It is concluded that the higher sustained CNS levels of 2B3-201 compared to free MP contributed to the increased efficacy in attenuating the MRI measures of neuro-inflammation. This was also in the context of an improved safety profile, as a result of drug encapsulation. The CNS-targeted anti-inflammatory agent 2B3-201 has therapeutic potential in human ALS.

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P317   INHIBITION OF PROTEIN AGGREGATION IN ALS

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Keywords: SOD-1, transgenic mice, stem cell-derived motor neurons, therapeutic

Background: To test the role of protein aggregation in motor neuron degeneration and cell death linked to ALS, we have evaluated a novel inhibitor of the aggregation of mutant superoxide dismutase (SOD-1). This compound, named molecular tweezer, or tweezer is a lysine-specific inhibitor of aggregation.

Methods: Stem-cell-derived motor neurons in cell culture were treated with the molecular tweezer CLR01 and survival and formation of protein aggregation in the cytosol were evaluated. In parallel, we treated mutant G93 SOD-1 transgenic mice with the tweezer and are following motor function, animal survival and motor neuron degeneration in these mice.

Results: Using stem-cell-derived motor neurons that express mutant SOD-1 as a disease model, we show that the molecular tweezer prolongs survival in this model system. Transgenic mice expressing mutant G93A exhibit preserved motor function and strength throughout their lifespan. At the time of this initial submission, we do not have sufficient data to analyze mouse survival rates.

Discussion: In summary, the molecular tweezer shows promise as a protein inhibitor in models of familial ALS linked to SOD-1 and may be useful as a research tool that helps understand disease mechanisms in ALS.

P318   TARGETED GENOME EDITING FOR DEVELOPING NOVEL THERAPEUTIC APPROACHES FOR SMA

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Keywords: SMA, iPSCs, genome editing

Background: Spinal muscular atrophy (SMA) is a severe autosomal recessive genetic motor neuron disease and the leading genetic cause of infant mortality. A possible strategy for treating patients with SMA consists of using genetically corrected induced pluripotent stem cells (iPSCs) for autologous cell therapy. The genetic correction can be achieved by direct genome editing using different strategies including site-specific nucleases (TALENs).

Objectives: To describe the genetic correction of human SMA-induced pluripotent stem cells (iPSCs) using site-specific nucleases TALEN.

Methods: Using a non-viral process, we produced and characterized iPSCs from human SMA fibroblasts and healthy subjects. As a first strategy, we employed a SMN2 sequence-specific oligodeoxynucleotides to direct the exchange of a T to C at position $+6$ of exon 7 in iPSC. This allows for the modification of SMN2 to a more SMN1-like sequence. In the second approach, we design a pair of TALENs for SMN2 genomic loci spanning the region flanking the same nucleotide. This method allows for the production of a double-strand break in the region. To complete the genetic correction process, we created a Piggy Back donor plasmid to correct the mutation by homologous recombination.

Results: Using defined methods, we successfully isolated SMA and WT iPSC subclones that were free from vectors and exogenous sequences. We were able to correct iPSC lines through a targeted gene correction approach with single-stranded oligonucleotides demonstrating the ability to isolate iPSC clones in which SMN2 functions as a SMN1-like gene. While motor neurons from uncorrected SMA-iPSCs reproduced disease-specific features, genetically corrected motor neurons showed phenotypic improvement. We synthesize a TALEN pair, and we were able to demonstrate its ability to cut into the target region a SMN2 plasmid. The co-trasfection of TALEN pair and the donor plasmids in SMA iPSC is ongoing.

Discussion and conclusion: Our results suggest that creating genetically corrected SMA-iPSCs could represent a viable cell source for therapeutic transplantation in SMA patients.

P319   NEW SYNERGISTIC GENETIC TREATMENT EXTENDS SIGNIFICANTLY DELAYS SYMPTOM ONSET AND PROLONGED SURVIVAL IN ALS MICE

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Keywords: gene therapy, glutamate uptake, NRF2

Background: Astrocyte activation occurs in response to central nervous system (CNS) insult and is considered a double-edged sword in many pathological conditions. Furthermore, we have previously described a reduced astrocytic glutamatergic and trophic response to activation. Here, we selected three pathways severally affected in ALS. For each pathway, we selected a single key gene that could affect the entire pathway. EAAT2, the major astrocytic glutamate uptake transporter, can reduce the synaptic glutamate availability, GDH2 converts glutamate into α keto glutarate in the metabolic pathway, thus depleting glutamate bio-availability, and NRF-2 is a major transcription factor in the cellular anti-oxidant response.

Results: In a mouse model of ALS (SOD1^{G93A}), intra-cisternal and intra-muscular injections of three lenti-viral constructs delayed body weight loss, preserved reflex score and motor performance, significantly delays symptom onset and prolonged survival by 120% and 136% from symptom onset in male and female ALS mice, respectively. Treatment of ALS mice with each of the genes individually had little effect.

Conclusions: Our approach to increase the anti-oxidant response in combination with reducing the glutamate excito-toxic levels in the central nervous system as well as with depleting the systemic glutamate bio-availability has proven to be a very effective therapeutic strategy in the ALS mouse model. We hope that our study might provide a novel strategy to slow disease progression and alleviate symptoms of patients suffering from ALS.

P320   THE ROLE OF STABILIZED NEUROPEPTIDES DERIVED FROM HYPERIMMUNE CAPRINE SERA (HICS) IN MOTOR NEURON DISEASE – IMPLICATIONS FOR A NOVEL THERAPEUTIC STRATEGY IN ALS PATIENTS

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Keywords: human, neuropeptide, biomarker

Objectives: The objective of the study was to determine whether targeting of the hypothalmo-pituitary-adrenal axis at a specific site using novel stabilized neuropeptides could elicit measurable efficacy in patients from a multi-center open-label prospective ALS study conducted up to 12 months in duration.

Recently, HCIS has exhibited evidence of neuroprotection, neuroregeneration and abrogation of pro-inflammatory responses in several in-vivo animal models of neurodegenerative disease and in two separate human phase II double-blind placebo clinical trial in secondary progressive multiple sclerosis and diffuse systemic sclerosis respectively.

Method: A single-arm multi-center open-label study up to 12 months was conducted in 21 subjects with definite ALS (according to the E1 Escorial criteria) received a daily s.c. dose of 1ml (4.5mg/ml) of HICS. The primary intention-to-treat analyses were ALSFRS-R and survival. Secondary outcomes were ALSAQ-40, Jablecki score, FVC, muscle strength, BMI, safety and tolerability. The M:F ratio was 9:1 (10% bulbar: 90% limb onset). In summary, improvement or stabilization in the ALSRFS-R was noted in the group of patients studied, with the majority having been treated for > 6 months. Patients showed a significant improvement in ALSFRS-R, ALSAQ-40, and in ALS scores of Jablecki (8.2%, p < 0.05), muscle power and lung function (FVC) during the study period were also noted. No adverse events were recorded during the entire duration of the study using HICS.

Results: The mechanism of action was investigated in the ALS cohort from serum taken from the patients pre- and post-treatment with HICS. Multiple micro-RNAs previously implicated in the pathogenesis of ALS, together with several candidate neuropeptides and pNFH change, were determined and related to clinical outcome. The results were compared to normal, no neurological disease, controls (n = 10) and patients from a phase II secondary progressive MS double-blind clinical trial (n = 20) who were treated with HICS. The groups acted as controls to elucidate whether a definable prognostic marker could be identified specific to the ALS cohort. These studies together with results from two independent SOD1^{G93A} mouse studies using HICS have allowed progress in the development of a rationale for further work in cadaveric human brain tissue taken from ALS patients where the various studies have allowed us to focus on mechanisms of neurotoxicity in ALS. The results of which will be available in Q4 of this year.

Discussion: HICS showed efficacy in humans with ALS with no safety concerns or adverse event recorded. This confirmed the safety profile of the drug once again as seen in two separate phase II clinical trials recently completed. The results of the open-label study in ALS are certainly encouraging but will need to be confirmed in a randomised placebo-control trial in the future.

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