Introduction

The Motor Neurone Disease Association, in collaboration with the International Alliance of ALS/MND Associations, welcomes you to Chicago for this year's International Symposium on ALS/MND. We are warmly welcomed by our hosts at the Les Turner Foundation as the symposium returns to Chicago for the first time in 16 years.

This year the programme offers plenty of new perspectives and insights into our understanding of the disease, including the C9ORF72 gene and frontotemporal dementia. The Programme Committee, chaired by Prof Wim Robberecht, has compiled a thought-provoking and varied platform programme reflecting some of these exciting new developments.

Joint opening and closing plenary sessions reflect on the risk factors of the disease and the challenges of translating knowledge to treatment. Parallel scientific and clinical sessions will run for the remainder of the symposium exploring a wide variety of topical themes in more detail, from target pathways, disease models and biomarkers, through to cognitive changes, multidisciplinary management and clinical trials.

This year, once again, we have a large number of high quality poster presentations, discussing novel ideas that promise to give us two sessions of riveting and exciting debate, along with furthering the international exchange of knowledge.

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SESSION 1 JOINT OPENING SESSION

C1 RISK FACTORS AND ALS: NATURE, NURTURE, AGE AND LUCK

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Keywords: genetics, epidemiology, risk factors

There are three parts that together cause any kind of illness or disease: the genes we carry, what happens to us during our lifetime, and randomness. This must also be true for motor neuron diseases like ALS, and trying to understand what the different underlying factors are has always been a major goal for ALS research.

At the moment, there is no good reason to think that anything we can do or that happens to us increases our risk of ALS, except for growing older - we know that it is more likely to affect people in their 50s to 70s. At least in some cases, the genes we carry also increase our risk. But there is a lot we still do not definitely know: does any aspect of lifestyle (for example smoking or exercise) increase risk? Is everyone with ALS predisposed from birth to develop it? If we all lived to 200, would we all develop ALS? Does ALS ever occur randomly, for no reason at all?

The answers to these questions are important because they would let us design new treatments and avoid behaviour that increases risk, but perhaps most importantly, because they would let us answer one of the first questions someone with ALS asks: "Why me?".

In the last few years, we have made a lot of progress in understanding why some people develop ALS and others do not. In this talk I will explain what we know, what we want to find out, and where we need to go from here.

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SESSION 2A RNA & PROTEIN DYSREGULATION

C2 CELLULAR IMPLICATIONS OF RNA REPEATS IN MYOTONIC DYSTROPHY

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Neuromuscular diseases Myotonic Dystrophies type 1 and type 2 (DM1 and DM2) are caused by unstable CTG and CCTG repeat expansions. In DM1, the expansion of CTG repeats occurs in the 3' UTR of the gene coding for Dystrophia Myotonica-Protein Kinase. Patients with DM2 contain CCTG expansion in the intron 1 of the gene coding for Zinc Finger Protein 9 (ZNF9). Whereas CTG and CCTG expansions are located in separated genes, coding for proteins with different functions, they cause similar diseases characterized by myotonia, muscle weakness, cardiac arrhythmias and neurodegeneration. CTG and CCTG expansions cause similar phenotypes due to accumulation of the mutant RNA CUG and CCUG repeats which are toxic for cellular functions. The toxicity of CUG/CCUG repeats is associated with alterations of RNA-binding proteins, which bind to the mutant RNAs. CUGBP1 and MBNL1 are the best characterized RNA-binding proteins targeted by RNA repeats. It has been shown that RNAs, containing long repeats, are very stable. The reduced degradation of mutant CUG/CCUG repeats leads to the aggregation of these RNAs. CUG and CCUG aggregates bind to RNA-binding proteins, such as MBNL1, reducing its availability for the normal cellular functions. The mutant RNA repeats outside of the aggregates stabilize and increase the levels of CUGBP1. The alterations of CUGBP1 and MBNL1 disrupt stability, splicing and translation of many RNAs in DM cells. Despite similarities of the toxic effects of the mutant CUG and CCUG repeats, there are also differences, specific for each disease. The mutant CUG repeats change several signaling pathways. Long CUG repeats increase the double-stranded RNA-activated protein kinase PKR. One of the substrates of PKR is eukaryotic initiation translation factor 2 alpha. Elevation of PKR in DM1 reduces the activity of eIF2 alpha affecting protein translation. CUG repeats also reduce cyclin D3, an important regulator of the cyclin-dependent kinases. Since cyclin D3-cdk4/6 signaling is involved in the regulation of cell proliferation and differentiation, the disruption of this signaling pathway causes a delay of DM1 myogenesis. The mutant CCUG repeats target the 20S proteasome. As the result, the stability of many proteins is increased in DM2 cells. It has been shown that CCUG RNA reduces the protein levels of ZNF9. One of the functions of ZNF9 is the regulation of translation of mRNAs, which contain a terminal oligopyrimidine tract (TOP). Because TOP-containing mRNAs encode predominantly components of the translation machinery, the rate of global protein synthesis is reduced in DM2. This pathway seems to be responsible for muscle atrophy in DM2. Thus, the molecular mechanisms by which the mutant RNA repeats cause DM pathology are complex and are associated with the disruption of many biological processes.

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C3 STRENGTHENING THE ARGUMENT FOR THE ROLE OF RNA METABOLISM IN ALS

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Keywords: RNA metabolism, RGNEF, p62

Background: A growing body of evidence now supports the hypothesis that ALS is a disease of the dysmetabolism of RNA (1–2). Several RNA-binding proteins (RBPs) are known to form neuronal cytoplasmic inclusions (NCIs) within spinal motor neurons (MNs). These include Rho guanine nucleotide exchange factor (RGNEF) (3), TAR-DNA binding protein of 43 kDa (TDP-43), and fused in sarcoma/translocated in liposarcoma (FUS/TLS). Interestingly, each of these proteins is known to bind low molecular weight neurofilament (NFL) mRNA and has the potential to regulate its stability.

Objectives: Our aim in this study was to provide strength to the hypothesis that an alteration in RNA metabolism is involved in ALS pathogenesis. We hoped to provide evidence that each of these RBPs (RGNEF, TDP-43, FUS/TLS) have the ability to interact with one another, as well as with markers of proteasomal degradation. Further, we aimed to understand whether RNA is sequestered into RBP NCIs.

Methods: The work presented here is based on immunofluorescent staining and confocal microscopy as well as co-immunoprecipitation and SDS-PAGE experiments. Sporadic ALS cases with no known mutations were used for the co-localization studies. Syto 14 was used as a marker of RNA-containing granules (4). Co-immunoprecipitation experiments were performed using lysates of a stable HEK293T cell line that overexpresses RGNEF.

Results: RGNEF, TDP-43 and FUS/TLS are each able to form morphologically diverse NCIs in ALS MNs, and are each able to co-localize with markers of proteasomal degradation. Further, each of the RBPs is able to co-localize with one another, an interaction that was also confirmed with co-immunoprecipitation and SDS-PAGE studies. Finally, these RBP-containing NCIs do not contain RNA, while the RBPs do seem to be present within RNA granules when not abnormally localized within NCIs.

Discussion and conclusions: Our data suggest that there are several proteins involved in the pathogenesis of ALS, and illustrates the danger in the belief that ALS is a disease of a singular RBP. Interestingly, these protein interactions seem to converge in one area of cell metabolism: RNA processing. Specifically, each of these proteins can bind to

and regulate the stability of NFL mRNA, the misregulation of which is known to lead to the classical ALS neurofilamentous inclusions.

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C4 CONVERGENT ROLES OF FUS/TLS AND TDP-43 IN PROCESSING RNAS WITH LONG INTRONS

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Keywords: RNA processing, FUS/TLS, TDP-43

Background: FUS/TLS and TDP-43 are RNA/DNA-binding proteins integrally involved in amyotrophic lateral sclerosis (ALS). We previously identified the RNAs bound and affected by TDP-43 in the mouse brain. Reduction of TDP-43 in the adult nervous system altered splicing of >900 pre-mRNAs and revealed an essential role for TDP-43 in sustaining the levels of long intron-containing transcripts that are important for neuronal function. Like TDP-43, FUS/TLS has been proposed to participate in several steps of RNA processing, however the precise role(s) of FUS/TLS in RNA metabolism regulation have not been determined. Since mutations in either TDP-43 or FUS/TLS cause a similar disease phenotype, we anticipate that the RNA-targets affected by both TDP-43 and FUS/TLS may be the most relevant for disease.

Objectives: To provide a systematic comparison of the binding patterns and roles in gene regulation for TDP-43 and FUS/TLS. To determine if TDP-43-FUS/TLS overlapping mRNA targets are altered in human neurons and in motor neurons of ALS patients.

Methods: We have used cross-linking immunoprecipitation CLIP-seq to identify RNAs bound by FUS/TLS in mouse and human brain. We have determined the effects of FUS/TLS loss of function on RNA expression and splicing patterns by using high-throughput sequencing of cDNA (RNA-seq) and splicing-sensitive arrays. We have assessed RNA alterations following TDP-43 and FUS/TLS depletion in human neurons differentiated from human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. We assayed individual motor neurons in autopsy samples from ALS patients by co-labeling for TDP-43 and proteins encoded by long pre-mRNAs whose maturation is dependent on TDP-43 and FUS/TLS.

Results: We have identified extensive FUS/TLS binding on >5,500 pre-mRNAs in mouse and human brain, primarily through a GUGGU-binding motif. A characteristic sawtooth-like binding pattern was observed, supporting co-transcriptional deposition of FUS/TLS. Depletion of FUS/TLS altered levels or splicing of >960 mRNAs, most of which are distinct from the RNAs whose maturation is dependent on TDP-43. Nonetheless, common targets reduced upon depletion of either TDP-43 or FUS/TLS in mouse brain and primary human neurons differentiated from stem cells were RNAs encoding proteins essential for neuronal integrity and that are transcribed from genes with exceptionally long introns. Two of these, KCNIP4 and parkin, were found to be significantly reduced in TDP-43 aggregate-containing motor neurons in sporadic ALS patients.

Discussion and conclusions: This study identifies convergence of the TDP-43 and FUS/TLS pathways in the regulation of a subset of transcripts that contain exceptionally long introns and encode protein products crucial for normal neuronal function. In sporadic ALS patients, cytoplasmic mis-accumulation of TDP-43 is accompanied by loss of proteins encoded by long pre-mRNAs pointing to a pathway underlying motor neuron death in ALS from misregulation of TDP-43 or FUS/TLS.

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C5 ROLE OF POST-TRANSLATIONAL MODIFICATIONS IN NUCLEAR-CYTOPLASMIC LOCALIZATION OF FUS AND ALS6-CAUSING MUTANTS

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Keywords: FUS, postranslational modification, ALS pathogenesis

Background: Mutations in fused in sarcoma/translated in liposarcoma (FUS/TLS) cause a familial form of ALS (ALS6). FUS is a DNA/RNA-binding protein with functions in transcription, RNA splicing, microRNA processing and RNA transport, which require shuttling between the nucleus and cytoplasm. FUS is mainly found in the nucleus, but mutants accumulate in the cytoplasm and form inclusions in motor neurons. Interestingly, FUS-positive inclusions are found in sporadic ALS, suggesting a common pathogenic pathway involving mislocalization of FUS. Asymmetric arginine methylation and phosphorylation are post-translational modifications known to affect trafficking of proteins across the nuclear membrane. Therefore, we have been examining the role of these modifications to distribution of both wild type and mutant FUS.

Objectives: 1) To determine how PRMT1-mediated asymmetric dimethylation of arginine residues affects the distribution of ALS6-causing FUS mutants in motor neurons. 2) To determine how activation of PKC-mediated phosphorylation of WT and mutant FUS affects their distribution. Others have shown that expression and activity of PKC, in particular PCKβ, is increased in ALS spinal cord, and that phosphorylation of FUS by PCKβII delays its proteasomal degradation.

Methods: Dissociated cultures of murine spinal cord are matured for three weeks. Human WT or ALS6-causing

mutants (flag or eGFP-tagged) are expressed in motor neurons by intranuclear microinjection of expression plasmids. Asymmetric arginine methylation was prevented by treatment with the methylase inhibitor, AdOx, or by expressing shRNA for PRMT1. To activate PKC, cultures were treated with phorbol-12-myristate-13-acetate (PMA).

Results: 1) Mutant FUS accumulated in the cytoplasm of motor neurons, shortened mitochondria, and gradually formed inclusions over a period of one week. Inhibiting methylation, maintained nuclear localization. Of note, PRMT1, the major enzyme catalyzing asymmetric arginine dimethylation in mammalian cells, mislocalized with mutant FUS. 2) In cultures treated with PMA, endogenous murine FUS left the nucleus and distributed throughout the cytoplasm within 2 hrs, appearing to concentrate in synaptic regions. Current studies are defining the isoform of PKC and phosphorylated epitopes on WT FUS, as well as the effect of activating PKC on disease-causing mutants and the ability of specific PKC antagonists to restore normal distribution.

Conclusions: Both asymmetric arginine methylation and PKC-mediated phosphorylation have major effects on localization of FUS. Inhibiting methylation retains the nuclear localization ALS-causing FUS mutants, preventing their accumulation in the cytoplasm and formation of abnormal structures. The methylating enzyme, PRMT1, also mislocalizes with FUS, implicating loss of nuclear functions of PRMT1, including control of gene transcription, in ALS pathogenesis. Activation of PKC promotes cytoplasmic distribution of FUS, consistent with its role in neuronal plasticity. Since PKC is highly activated in ALS, phosphorylation of WT FUS may play an important role in pathogenesis of sporadic ALS and inhibition of specific PKC isoforms might be exploited therapeutically.

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C6 ARGININE METHYLATION MODULATES NUCLEAR IMPORT OF FUSED IN SARCOMA (FUS)

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Keywords: FUS, transportin, arginine methylation

Fused in sarcoma (FUS) is a RNA-binding protein that regulates transcription and splicing. FUS carries a proline-tyrosine nuclear localization signal (PY-NLS) and is imported into the nucleus via the import receptor Transportin (TRN). Defects in nuclear import of FUS have been implicated in neurodegeneration, since mutations in the PY-NLS of FUS cause amyotrophic lateral sclerosis (ALS) and cytoplasmic FUS inclusions are a pathological hallmark in a subset of frontotemporal lobar degeneration (FTLD) patients. Indeed we have shown previously that age of onset and the degree of cytoplasmic missorting of individual FUS mutations negatively correlate (1,2). We now demonstrate that arginine methylation modulates nuclear import of FUS. Chemical inhibition of methylation or knockdown of protein arginine methyltransferase 1 (PRMT1) rescues TRN-mediated nuclear import defects of ALS-associated FUS mutants. The unmethylated arginine-glycine-glycine (RGG) domain preceding the PY-NLS of FUS binds tightly to TRN and arginine methylation in this domain reduces the affinity to TRN. Our results reveal a novel concept of TRN-cargo recognition and implicate arginine methylation in the pathogenesis of FUS-associated diseases.

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C7 DYSREGULATED MICRORNAS IN THE PATHOGENESIS OF ALS

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Keywords: microRNA, RNA, regulation

Background: Genome-encoded microRNAs are negative posttranscriptional regulators, contributing to a wide variety of biological processes in health and disease. ALS-causing mutations, recently discovered in genes encoding for RNA-binding proteins, suggest that dysregulation of RNA-related processes are fundamental in ALS pathogenesis.

Objective: We tested the magnitude, mechanism and functional consequences of dysregulation in microRNA expression in sporadic and familial forms of ALS, by studies of microRNA expression in motoneurons of ALS patients, microRNA molecular biology approaches and mouse genetics.

Methods and results: We quantified microRNA expression in samples extracted from laser-capture microdissected spinal motoneuron punches of ALS patients who had met El Escorial criteria for definite ALS and of controls that were not reported to suffer from neurodegeneration. Tissue collections were completed within 4–6 h of death and RNA quality was assessed using microelectrophoresis on an Agilent 2100(1). Quantification of 667 microRNAs performed by using microRNA TaqMan® qPCR Megaplex pool arrays. This study revealed 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. This observation was substantiated by in-situ hybridization, which revealed comparable downregulation of microRNAs in patient tissue, relative to control.

Next, we transfected NSC-34, a motoneuron hybridoma-cell line, with vectors for expression of ALS-causing mutant forms of FUS and TDP-43, namely FUS495X, FUSR521G, TDP-43A315T or TDP-43M337V. In culture, mature microRNAs were also downregulated, reminiscent of the observations in human ALS patients. Intriguingly, the levels of cognate pre-microRNA precursors were in fact upregulated. These observations suggest that canonical microRNA bioprocessing is disrupted at the level of Dicer1 activity, the RNase type III

responsible for processing of pre-microRNA precursors into their mature functional form (2).

To address functional consequences for loss of Dicer1 and microRNAs in motoneurons, we established a mouse line, wherein Dicer1 conditional allele was mated to a Cre-recombinase transgene, driven by a cholinergic-specific promoter. Consistent with the data from human ALS patients, loss of Dicer1 and microRNAs activity in mice resulted in neurodegeneration of spinal motoneurons and in denervation-dependent muscle atrophy (3).

Conclusions: Dysregulated microRNAs provide new mechanistic insight into ALS pathogenesis. This novel microRNA-based mechanism is probably involved in several forms of ALS and may be of therapeutic usage in the future, if potent molecules modulating microRNA maturation or activity could be developed.

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C8 MUTATIONS IN PROFILIN 1 CAUSE FAMILIAL ALS

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Keywords: exome sequencing, mutations, insoluble aggregates

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disorder resulting from motor neuron death. Approximately 10% of cases are familial (FALS), typically with a dominant inheritance mode. Despite numerous advances in recent years, genetic etiology of all FALS cases is not known. Here we show that mutations within the profilin 1 (PFN1) gene can cause FALS. PFN1 is critical for monomeric (G)-actin conversion to filamentous (F)-actin. Exome sequencing of two large ALS families revealed different mutations within the PFN1 gene. Additional sequence analysis identified 4 mutations in 7 out of 274 FALS cases. Cells expressing PFN1 mutants contain ubiquitinated, insoluble aggregates that in many cases contain the ALS-associated protein TDP-43. PFN1 mutants also display decreased bound actin levels and can inhibit axon outgrowth. Furthermore, primary motor neurons expressing mutant PFN1 display smaller growth cones with a reduced F-/G-actin ratio. These observations further document that cytoskeletal pathway alterations contribute to ALS pathogenesis.

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SESSION 2B COGNITIVE CHANGE

C9 ALS/FTD: CORRELATIONS WITH PATHOLOGY

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Amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND) has been clinically linked with dementia since the 1950s. Pathologic linkage between ALS and frontotemporal dementia (FTD) was reported almost 40 years later, and finally the two were linked molecularly six years ago, when TAR DNA-binding protein of 43kD mw (TDP-43) was reported to be the major protein component of the insoluble inclusions in both frontotemporal lobar degeneration with ubiquitin positive, tau and alpha-synuclein negative inclusions (FTLD-U, now called FTLD-TDP) and ALS. Mutations in *TARDBP*, the gene encoding TDP-43, have been reported, predominantly in ALS but also in FTLD-TDP, providing a genetic link. Mutations have subsequently been found in both FTLD-TDP and ALS in *VCP* and *C9ORF72*. TDP-43 molecular pathology links FTLD-TDP with sporadic and non-SOD1 familial ALS (fALS) and together these are now called TDP-43 proteinopathies. There are four sub-types of TDP pathology, types A, B, C, and D, and they correlate with specific clinical and genetic profiles. These sub-types are determined by the morphology, predominance, and distribution of the various TDP-43 immunopositive insoluble aggregates – neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs), and dystrophic neurites (DNs). TDP type A has NCIs, NIIs, and short neurites in upper cortical layers, is associated with *GRN* and sometimes *C9ORF72* mutations, and ALS is sometimes present. TDP type B has NCIs in all cortical layers, is often associated with ALS, and may be associated with *C9ORF72* mutations. TDP type C has long neurites in upper cortical layers, no NIIs, ALS is absent, and so far there are no known associated genetic mutations. Type D has predominantly NIIs in upper cortical layers, no DG inclusions, and is associated with *VCP* mutations and inclusion body myositis or ALS, Paget's disease of bone, and frontotemporal dementia. A minority of FTLD-U/ALS cases is TDP-43 negative, and include FUS proteinopathies (FTLD-FUS, which includes atypical FTLD-U, neuronal intermediate filament inclusion disease, and basophilic inclusion body disease) and fALS related to *FUS* mutations, both of which are immunopositive for FUS, FTLD-UPS (exemplified by FTLD cases with *CHMP2B* mutations), and FTLD without inclusions or FTLD-ni. Interestingly, fALS cases with *SOD1* mutations are also negative for TDP-43, although mutated *SOD1* has been shown to interact with TDP-43. So far, mutations in *GRN* and *CHMP2B* have been found in only FTLD and mutations in *SOD1*, *FUS*, and several other genes have been found in only ALS. There are reports of combined TDP-43 and FUS pathology in both FTLD-TDP and ALS, although this is currently controversial. Lastly, cases with *C9ORF72* repeat expansion also have unique pathology in the cerebellum that is p62 and ubiquitin positive and TDP-43 negative, suggesting that another protein remains to be identified in this disorder.

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C10 THE HETEROGENEITY OF COGNITIVE IMPAIRMENT IN ALS: SUBPHENOTYPES ON THE ALS-FTD CONTINUUM

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Keywords: cognition, subphenotypes, frontotemporal dementia

Background: Cognitive research in ALS has focused on finding whole group differences between patients and healthy controls. More recently, studies have investigated the heterogeneity of impairment within ALS by using single case analysis, either by classifying patients according to the severity of their impairment, or uncovering ALS-subgroups with qualitatively distinct deficits. Here we investigate the relationship between executive and language deficits in ALS by using executive tasks sensitive to the dysfunction of the orbitofrontal cortex, a region showing early atrophy in behavioural variant FTD, and language tasks which evaluate semantic functions affected more in temporal variant FTD.

Objectives: To characterize the heterogeneity of cognitive and behavioural impairment in a sample of ALS-patients, as well as to determine whether language and executive changes occur independently or simultaneously in individual patients.

Methods: Thirty-seven, non-demented ALS patients were administered a comprehensive battery of neuropsychological tests, including tests of orbitofrontal function and semantic association. Furthermore, the battery covered a traditional language (naming) task as well as tasks tapping into 'dorsolateral' executive functions, episodic memory, visual and behavioural functions. Abnormality was established if test performance was more than 2 standard deviations above or below the mean of a healthy control group, matched for age, sex and education. Patients were subsequently classified into the following subgroups: intact functions (ALS-pure) or with executive (ALS-Ex), non-executive (ALS-NECI) or behavioural (ALS-bi) impairment.

Results: Twenty-one patients were identified as ALS-Ex (56.8%). Seven patients (18.9%) showed isolated executive impairment, and another two (5.4%) displayed additional visual but not language dysfunction. Hence, nine patients (24.3%) showed executive impairment without language involvement. Fourteen of the 21 ALS-Ex patients exhibited

dysfunction in at least one other domain (66.7%). Of these Executive + patients, the vast majority (12 patients, 85.7%) showed additional language dysfunction, comprising 32.4% of the total sample. Seven patients (18.9%) were categorized as ALS-NECI, and exhibited language dysfunction without executive changes. Only one patient (2.7%) classified as ALS-bi, showing isolated behavioural impairment; four ALS-Ex patients (10.8%) showed additional behavioural impairment. Eight patients (21.6%) were cognitively and behaviourally normal.

Discussion and conclusions: The present results reveal qualitatively distinct subtypes in classical ALS. The subphenotypes appear to lie on the ALS-FTD continuum and comprised most frequently of cases displaying a mixed profile of features of both behavioural and temporal FTD variants, followed by cases in which either executive or language dysfunction was present in isolation.

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C11 A NATIONAL MULTICENTER STUDY OF REGIONAL AND GENDER DIFFERENCES IN FRONTOTEMPORAL DISEASE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FTD, gender, cognition

Background: A large scale international multicenter study of Frontotemporal Dementia (FTD) found sex differences in FTD subtype incidence rates (1). Neuroimaging studies evidence a predilection for many language processing tasks that is left hemisphere lateralized in males while bi-hemispheric in females. Women display relative advantages for aspects of language processing, while men perform better at visuospatial processing (2,3,4). We hypothesized that gender differences would exist in emerging language processing declines in ALS, with greater associations for females than males between 1) left and right hemisphere mediated tasks and 2) frontal and temporal cortical-mediated tasks.

Objectives: To investigate regional and gender differences in prevalence rates and pattern of cognitive (ci) and behavioral (bi) impairment in ALS.

Methods and materials: 110 subjects (55 M) from 14 ALS clinics were evaluated cross-sectionally with the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes (PSFTS). Gender and regional groups were equivalent for education and IQ. Regional groups were age equivalent, while females were significantly older (male $M = 56.3$, female $M = 60.6$; $p = 0.001$).

Results: Prevalence rates of ci and bi were statistically equivalent among rural, suburban and urban subgroups. Females evidenced significant strengths in letter fluency (LF) ($p = 0.017$) and category fluency (CF) ($p = 0.019$) and limitations in configurational processing ($p = 0.032$) in comparison to males. Correlational patterns of regional cognitive findings suggested greater frontal cortical involvement in the rural sample (attention and comprehension $p = 0.036$, LF and CF $p = 0.000$). Females demonstrated more bi-hemispheric involvement in comparison to more left hemispheric involvement for males (LF and 2-D constructions $p = 0.035$).

Discussion and conclusions: ALS FTD prodrome regional prevalence differences appear insignificant and multifactorial, while consistent with the toxicity model implicating pesticides in frontal lobe change (5). This preliminary study warrants further study. Female gender potentially masks the FTD prodrome due to bilateral distribution of language processing, requiring assessment of right hemisphere-mediated capacities to detect.

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C12 FUNCTIONAL RATING SCALES IN AMYOTROPHIC LATERAL SCLEROSIS: APPLICABILITY OF THE FRONTOTEMPORAL DEMENTIA RATING SCALE

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Keywords: disease progression, ALSFTD, ALSFRS-R

Background: ALS shares significant clinical, genetic, pathological and neuroimaging overlap with frontotemporal dementia (FTD), a neurodegenerative condition characterised by significant atrophy of the frontal and anterior temporal lobes. Importantly, similar patterns of behaviour (eg, apathy) and cognitive impairments (eg, executive and language deficits, impaired social cognition) exist across both conditions. Although ALS and FTD lie within the same disease continuum, differing functional rating scales are used, and there is an increasing need of a common disease staging scale that could be used to match both patient groups in comparative studies. The ALSFRS-R, which assess for limb and bulbar impairments as well as respiratory function, is used in ALS whereas the Frontotemporal dementia Rating Scale (FRS), assesses cognitive and behavioural symptom severity, is used in FTD. Recent findings in ALS suggest that changes in behaviour also have a significant impact on carer stress and burden. The FRS, therefore, may be a useful adjunct to the ALSFRS-R but has not yet been applied to a MND cohort to date.

Objectives: To investigate the utility of the FRS in an ALS population in New South Wales, Australia.

Method: The ALSFRS-R and FRS were obtained from a postal survey of carers/family members ($N = 130$) recruited with the support of the NSW MND Association of Australia. On the FRS, three categories were used: "mild", "moderate" and "severe". Measures of carer burden and mood symptomatology were also obtained.

Results: The ALSFRS-R (M = 30, SD = 9.3) but not the FRS ("mild" = 30.8%, "moderate" = 63.1%, "severe" = 6.2%) was significantly correlated with disease duration (months; M = 4.7, SD = 5.6) in ALS patients (26% bulbar onset; 74% limb onset). The FRS, in contrast, was not significantly correlated with the ALSFRS-R ($p > 0.10$). For example, a proportion of patients (12.5%) with mild physical symptoms (ALSFRS-R scores between 37 and 48) were rated as "severe" on the FRS. Conversely, 34.4% of patients who are severely physical impaired (e.g., ALSFRS-R scores below 24) showed only "mild" impairment on the FRS. Finally, the ALSFRS-R and FRS correlated with caregiver burden and stress, respectively ($p's < 0.01$).

Discussion: The ALSFRS-R and FRS are functional rating scales which measure motor, cognitive and behavioural symptoms, respectively. The two measures are unrelated but both are associated with important aspects of ALS symptoms, which are strongly correlated with caregiver burden and stress.

Conclusion: Functional rating scales are useful in determining disease staging and progression, particularly in the context of drug trials in ALS and its overlap with FTD. Current ALS measures alone do not encapsulate the range of symptoms which are observed in ALS. The FRS, therefore, may be a useful adjunct to the ALSFRS-R in the context of ALS and FTD disease continuum.

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C13 COGNITIVE AND BEHAVIOURAL DEFICITS DRIVE CORTICAL ATROPHY IN ALS WITHOUT ALS-FTD

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Keywords: voxel-based morphometry, cognitive deficits, behavioural changes

Background: Despite severe motor deficits, cortical motor cortex atrophy has not been consistently observed across ALS patients. It is still unclear why some ALS patients show cortical atrophy while others do not; one potential reason for this variability would be that behavioural and cognitive deficits drive the cortical atrophy in these patients. By applying the ALS Frontotemporal Dementia (ALS-FTD) consensus diagnostic criteria, we predicted that cortical atrophy would be only present in ALS patients with cognitive and behavioural deficits, even those who did not qualify for a diagnosis of ALS-FTD.

Objective: To characterize the patterns of brain atrophy in ALS patients with and without cognitive/behavioural deficits, in comparison to controls.

Methods: 57 participants (ALS = 21; ALS-FTD = 17; controls = 18) were included, following current ALS and FTD criteria. ALS patients were further sub-classified according to the Strong criteria (deficits in 2 non-overlapping behavioural domains or falling below 5th percentile on 2 executive tests) into ALS with cognitive/behavioural syndrome (ALS-plus group; n = 8) and ALS patients with no cognitive/behavioural deficits (ALS-pure group; n = 14). All patients undertook extensive neuropsychological (executive; memory; language; emotion processing) and neuropsychiatric (apathy; stereotypical behaviour; abnormal behaviour) assessments, and underwent a brain MRI on the same date. Voxel-based morphometry (VBM) analysis was conducted to establish patterns of brain atrophy using the FSL software package.

Results: There was a clear gradation of brain atrophy across patient groups. The ALS-FTD showed substantial atrophy (prefrontal cortex regions, motor cortex, ventromedial prefrontal cortex, bilateral temporal pole regions), which is similar to the pattern observed in FTD. The ALS-plus group showed less cortical atrophy than ALS-FTD, but still showed substantial atrophy in the motor and prefrontal cortex areas. Finally, the ALS-pure group showed only marginal cortical atrophy in motor cortex and prefrontal brain areas.

Discussion: Our results show a clear gradation in regions of brain atrophy for ALS pure, ALS-plus, and ALS-FTD patients. More importantly, our study shows that ALS-pure patients have minimal cortical atrophy, indicating that previous results showing substantial atrophy in the motor cortex might have been driven by the inclusion of ALS-plus patients.

Conclusions: Studies addressing the neural basis of ALS should consider the specific characteristics of ALS-plus patients, which could bias findings. The subtle presence of cognitive and behavioural deficits is indicative of an ALS-plus syndrome, which could drive the motor cortical degeneration. Future studies need to establish whether ALS-plus follows a dying forward pattern of disease progression, and whether ALS-pure originates in the spinal cord, which in turn would explain the absence of behavioural and cognitive deficits in ALS-pure patients.

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C14 STRUCTURAL CONNECTIVITY AND AMYOTROPHIC LATERAL SCLEROSIS AND FRONTO-TEMPORAL DEMENTIA – EVIDENCE FROM DIFFUSION TENSOR IMAGING

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Keywords: diffusion tensor imaging, ALS-FTD, cognitive impairment

Background: Recently a continuum between amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) is encouraged by pathological and genetic characteristics, further supported by frontotemporal atrophy and hypometabolis in ALS patients with dementia. To objectify overlapping cognitive and behavioral profiles is made possible in particular by more differentiated neuropsychological test batteries and evaluation criteria.

Objectives: To investigate extra-motor white matter (WM) integrity in ALS patients in order to identify defined patterns of WM microstructural changes related to cognition

Methods: We investigated 66 ALS patients in comparison to 33 age and sex-matched healthy controls (HC). The ALS-group was categorized and divided as follows: 1) non cognitively impaired ALS patients (ALSnci, N = 30); 2) cognitively impaired ALS patients (ALSci, N = 28, (1, 2); 3) ALS-FTD patients (N = 8;(3)).

Diffusion tensor imaging was used to investigate white matter integrity. Fractional anisotropy (FA) values were analysed by both a whole brain voxel-based approach utilizing tract-based spatial statistics (TBSS) and an analysis of region of interest (ROI) after parcellation. For group comparisons, analyses of variance (ANOVAs) were performed.

Results: Significant FA reductions outside the corticospinal tract were identified by group comparisons as follows: in ALSci vs. HC, in the body of the corpus callosum (BCC) and bilaterally in the corona radiate; in ALSci vs. ALScni, in the posterior part of the right inferior fronto-occipital fasciculus (IFOF); in ALSci vs. ALS-FTD, in the uncinate fasciculus, in the rostral parts of the IFOF, in the cingulum, and in the BCC, including the forceps minor.

Discussion and conclusions: There may be a correlation between extra-motor WM microstructural changes and cognition in ALS patients. In accordance with the recent findings (4), our results show a concentration of WM lesions in the frontal and temporal lobes. Overlapping findings (BCC, IFOF) may indicate a continuum between ALSci and ALS-FTD. Further investigations should identify and confirm patterns of WM damage that could predict the transition from ALSci to ALS-FTD. Future research should extend the analyses of structural connectivity to the dimension of behavior.

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SESSION 3A CELL STRESS MECHANISMS

C15 SELECTIVE AUTOPHAGIC DEGRADATION OF AGGREGATE-PRONE PROTEINS

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Autophagy is a catabolic mechanism of the cell that allows recycling of cytoplasmic organelles and macromolecules through their sequestration into double-membrane vesicles (autophagosomes) which fuse with lysosomes, leading to degradation of the sequestered biomolecules in the acidic interior of the lysosome. Long considered a non-selective process induced in response to cellular starvation, autophagy is now emerging as a highly selective quality control mechanism whose basal levels are important to allow cells to rapidly eliminate large unwanted structures such as aberrant protein aggregates, superfluous or damaged organelles and invading pathogens. We are just starting to unveil the regulation and mechanism of these selective types of autophagy, but what it is already clearly emerging is that structures targeted to destruction are accurately enwrapped by autophagosomes through the action of specific receptors and adaptors.

The ubiquitin-binding protein p62/SQSTM1 has been identified as a specific cargo receptor involved in selective autophagic degradation of intracellular aggregation-prone ubiquitinated proteins, a process termed aggrephagy. p62 interacts with the autophagosomal membrane protein Atg8/LC3 and thereby targets the ubiquitinated proteins for autophagic degradation. We have recently found that ALFY (autophagy-linked FYVE protein), a large PI3P-binding protein, is central to this selectivity. ALFY is recruited to intracellular inclusions and scaffolds a complex containing p62 and the autophagy effectors Atg5 and GABARAP (1, 2). Depletion of ALFY inhibits clearance of huntingtin aggregates, but has no detectable effect on the non-selective starvation-induced autophagy. Importantly, ALFY overexpression diminishes inclusion number and leads to neuroprotection in a neuronal and Drosophila model of Huntington's disease, indicating that ALFY mediates selective autophagy of aggregating proteins (2).

Prior to fusion with the lysosomes, autophagosomes fuse with endocytic vesicles and we have shown that the endosomal sorting complexes required for transport (ESCRTs) are required for autophagic degradation of aggregate-prone proteins (3). Interestingly, mutations in the ESCRT-III subunit CHMP2B are associated with frontotemporal dementia and amyotrophic lateral sclerosis, indicating that dysfunctional autophagy may underlie to the observed neurodegenerative phenotype seen in patients with CHMP2B mutations.

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C16 ER-GOLGI TRANSPORT IS A COMMON MECHANISM OF TOXICITY SHARED BY SOD1, TDP43 AND FUS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TDP43, FUS, ER-Golgi transport

Background: The pathogenic mechanisms triggered by TDP-43 and FUS in ALS are poorly understood. Previously we showed that mutant forms of both TDP43 and FUS trigger endoplasmic reticulum (ER) stress in cellular models of ALS. Induction of ER stress usually requires the accumulation of proteins within the ER. However, both TDP-43 and FUS lack a signal ER-targeting peptide and hence should not enter the ER. However, the failure of ER-Golgi transport can also trigger ER stress, due to the accumulation of secretory proteins within the ER. ER-Golgi transport is driven by proteins including COPII, Rab1 and dynein, which when impaired, will inhibit ER-Golgi transport. We previously showed that mutant SOD1 inhibits ER-Golgi transport, triggering ER stress, possibly by an aberrant interaction with COPII and dynein.

Objectives: The objectives of this study were to determine whether mutant TDP-43 and mutant FUS also impair secretory protein transport between the ER and Golgi, consequently inducing ER stress. We also examined whether mutant TDP43 or mutant FUS also interact with proteins involved in ER-Golgi transport.

Methods: $VSVG^{ts045}$ is widely used to examine ER-Golgi transport. Neuro2a cells were co-transfected with GFP-TDP-43 or HA-FUS and $VSVG^{ts045}$-mCherry at different time points. After transfection, immunocytochemistry and confocal microscopy were performed to examine ER stress by nuclear immunoreactivity to CHOP. The interaction of TDP-43 or

FUS with transport proteins were examined by immunoprecipitation, western blotting and immunocytochemistry.

Results: Using VSVG^{ts045}, both mutant TDP-43 and mutant FUS were found to inhibit ER-Golgi transport in Neuro2a cells. This inhibition of transport occurred prior to the induction of ER stress and was one of the earliest cellular events we detected after transfection. Mutant TDP-43 was found to physically interact and co-localize with both Rab1 and dynein, whereas mutant FUS was found to physically interact and co-localize with Rab1 and COPII, but not dynein. These findings suggest that the common mechanism of toxicity triggered by mutant SOD1, TDP-43 and FUS is the inhibition of ER-Golgi transport. The physical interaction between mutant SOD1, TDP-43 and FUS and proteins involved in ER-Golgi transport such as Rab1, could be the mechanism by which this inhibition occurs. Furthermore, overexpression of Rab1 rescued ER stress and the cytoplasmic translocation of mutant TDP-43 and mutant FUS, demonstrating a further link to disease.

Discussion and conclusions: These findings show that dysfunction of ER-Golgi transport is a common and early pathogenic mechanism triggered by SOD1, TDP-43 and FUS in ALS. Mutant SOD1, TDP-43 and FUS bound to several proteins involved in ER-Golgi transport, including Rab1, the only protein we examined which bound to all three. These data suggest that Rab1 plays a key role in the failure of ER-Golgi transport and pathogenesis in ALS.

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C17 REDUCTION IN THE AUTOPHAGY PROTEIN, BECLIN 1, ACCELERATES DISEASE PROGRESSION AND LEADS TO ACCUMULATION OF MUTANT SOD1 AGGREGATES IN A MOUSE MODEL OF ALS

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Keywords: autophagy, aggregates, SOD1 mutant

Background: A major known cause of amyotrophic lateral sclerosis (ALS) is mutations in the gene encoding copper/zinc superoxide dismutase (SOD1). Aggregates/inclusions immunoreactive for SOD1 are hallmarks of ALS caused by mutant SOD1s. To maintain protein quality in the cell, there are two main cellular pathways for degradation of misfolded proteins: the ubiquitin-proteasome system and the autophagy-lysosome system. Several *in vitro* studies have shown that mutant SOD1 aggregates can be degraded by autophagy. However, the functional importance of autophagy against SOD1 aggregates has not been tested *in vivo*.

Objectives: The aim of the present study was to address whether autophagy protects against mutant SOD1-linked ALS.

Methods: Since Beclin 1 is a crucial protein in the autophagy pathway, hemizygous SOD1^{G93A} mice were crossed with hemizygous Beclin 1 knockout mice to generate double hemizygous mice. Disease onset was defined as the time when mice reached peak weight before decline. The end-point was defined as the age at which a mouse was unable to right itself within 5 seconds after being pushed onto its side. Disease progression was defined as the period from disease onset to the end-point. The status of autophagy was assessed as the expression level of LC3-II, a marker for induction of autophagy. To elucidate the effects of autophagy on SOD1 aggregates, spinal cords from terminal mice were separated from detergent-insoluble fractions. The fractions were analyzed by Western blot using antibody against human SOD1.

Results: The time of onset was not altered in double hemizygous mice as compared with hemizygous SOD1^{G93A} mice. However, the lifespan was significantly shortened from 172 ± 6.1 days to 152 ± 5.8 days, representing a decrease of 8.7%. Reduction in Beclin 1 accelerated the disease progression by 35% from 49 ± 2.2 days to 31 ± 4.8 days. There was also a significant decreased level of LC3-II. Notably, further accumulation of SOD1 aggregates was observed in spinal cords of Beclin 1-reduced SOD1^{G93A} mice.

Conclusion: Beclin 1 dependent autophagy protects against disease progression, and regulates degradation of mutant SOD1 aggregates.

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C18 FAILURE OF AUTOLYSOSOME FORMATION RESULTS IN IMPAIRED AUTOPHAGY IN UBQLN2-LINKED ALS-FTD

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Keywords: FTD, dementia, UBQLN2

Background: Mutations in *UBQLN2* cause amyotrophic lateral sclerosis (ALS) and ALS with frontrotemporal lobe dementia (ALS-FTD). Pathological inclusions containing UBQLN2 are a common pathological feature in a wide spectrum of ALS and ALS-FTD, including *SOD1*-linked ALS. Recent findings have linked abnormalities in UBQLN2 to defects in protein degradation via the ubiquitin-proteasome system (UPS), abnormal protein aggregation and neurodegeneration. UBQLN2 sits at the crossroads of protein degradation through the UPS, and bulk lysosomal degradation via autophagy. Continuous turnover of intracellular components by autophagy is essential to preserve neuronal homeostasis. Alterations in autophagy have been proposed to contribute to pathogenesis in several neurodegenerative diseases including ALS. Although a defective UPS has been suggested to produce ALS-associated protein aggregates, recent studies have revealed a prominent role for autophagy. However, the precise mechanism behind autophagy malfunction in ALS is poorly understood.

Objectives: To further explore the pathogenic mechanism of UBQLN2-mediated ALS and ALS-FTD and the effect of mutant UBQLN2 on autophagy.

Methods: To study the effect of UBQLN2 mutations on autophagy, neuro-2a cells were transiently transfected with expression vectors containing wildtype (wt) UBQLN2, P497H-UBQLN2 or P506T-UBQLN2. For flow cytometry and imaging studies, cells were co-transfected with an autophagosome marker (GFP-LC3). Fourty-eight hours

post-transfection cells were collected using a BD LSRFortessa flow cytometer and analyzed using BD FACSDiva software. For imaging studies, cells were fixed 24 hours post-transfection, immunolabeled with anti-UBQLN2, anti-p62 or anti-LAMP1 antibodies and analyzed using a Zeiss LSM 510 Meta laser scanning confocal microscope. Endogenous LC3 and p62 turnover assay was performed using Western blotting according to standard protocols. Skin fibroblast cells derived from patients with ALS-FTD were used to confirm our findings.

Results: Using cellular models and cells derived from patients with ALS-FTD, we found that cells expressing mutant UBQLN2 accumulate autophagosomes and autophagosome precursors. Expression of mutant UBQLN2 leads to an accumulation of autophagosome-associated proteins, LC3 and p62. After autophagic induction, autophagosomes in mutant UBQLN2 expressing cells fail to mature into autolysosomes and degrade LC3 and p62.

Conclusions and discussion: These data shed light on the possible mechanism through which mutant UBQLN2 may be pathogenic by highlighting an effect on the autophagy pathway. Collectively, these data implicate UBQLN2 in autophagy, and suggest that impaired autophagy due to the failure of autolysosome formation is central to the pathogenesis of UBQLN2-linked ALS and ALS-FTD and may explain the pathology seen in ALS and FTD patients. This mechanism could accelerate the accumulation of the toxic aggregation-prone proteins in ALS, and impair essential regulatory functions of autophagy and the UPS. Hence, autophagy represents an attractive target for designing rational therapeutics in ALS and FTD.

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C19 PROPAGATED MISFOLDING OF SOD1 IN FAMILIAL AND SPORADIC ALS

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Keywords: copper-zinc superoxide dismutase, prion-like mechanisms, misfolding-specific antibodies

Background: Sporadic ALS without known genetic mutation is clinically identical to familial ALS, in which mutations in superoxide dismutase 1 (SOD1), TDP43 and FUS are causal. A consequence of SOD1 mutation and/or oxidation is a propensity of the protein to misfold and aggregate. Human wild-type (wt) SOD1 is known to co-aggregate with mutant SOD1 in familial ALS (FALS), in double transgenic mouse models, and in cell culture systems. However, the capacity of wtSOD1 to engage in serial prion-like activity is unclear, as is the genesis of wtSOD1 misfolding in TDP43 or FUS-associated ALS, and the potential relevance of this process to sporadic ALS (SALS).

Objectives: To molecularly dissect the effects of misfolded mutant or wild-type (wt)SOD1 on natively structured wtSOD1; to determine if cytoplasmic mislocalized TDP43 or FUS was associated with SOD1 misfolding; and to quantify wtSOD1 misfolding in FALS and SALS.

Methods and results: Transient transfection-driven expression of natural FALS SOD1 mutations G127X and G85R, or overexpression of wtSOD1, in human mesenchymal and neural cell lines induced misfolding of wild-type natively-structured SOD1, as indicated by: 1) acquisition of immunoreactivity with SOD1 misfolding-specific monoclonal antibodies (mAbs); 2) markedly enhanced protease-K (PK) sensitivity suggestive of structural loosening; and 3) non native disulfide-linked oligomer and multimer formation. Cytosolic mislocalizing mutations of TDP43 and FUS, and overexpression of wtTDP43, were also associated with SOD1 misfolding by immunocytochemistry and immunoprecipitation with SOD1 misfolding-specific mAbs. Culture media from cells transiently transfected with wild-type or mutant SOD1 induced misfolding of endogenous SOD1 when added to naive neuroblastoma cell cultures, and this process was stably propagated in serial passage. Nonspecific uptake of misfolded SOD1 was excluded by siRNA knockdown of SOD1 in the fresh recipient cells, indicating a requirement for endogenously expressed SOD1 as a substrate. The agent responsible for induction of misfolding was determined to be a misfolded SOD1 aggregate which pelleted by ultracentrifugation of 100,000 X g for 1 hr. Transmission of SOD1 misfolding *in vitro* was abrogated by extracellular pan- and misfolding-specific SOD1 antibodies. On quantitative immunoprecipitation with SOD1 misfolding-specific mAbs, misfolded wtSOD1 was found to constitute ~5% of total SOD1 in spinal cord samples from SOD1 familial FALS as well as sporadic ALS, which was PK sensitive compared to normal and disease controls.

Discussion and conclusions: wtSOD1 misfolding can propagate within and between cells, fulfilling prion-like activity similar to mutant SOD1. wtSOD1 misfolding may be a cause or consequence of mutant TDP43/FUS or wtTDP43 cytoplasmic mislocalization. A surprisingly large proportion of wtSOD1 in SALS was found to be misfolded by immunoreactivity and PK sensitivity. These data support the hypothesis that propagated misfolding of SOD1 participates in the pathogenesis of all types of ALS.

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SESSION 3B AUTONOMY AND DECISION MAKING

C20 ASSISTING PATIENT CHOICES: AUTONOMY, PATERNALISM, OR SOMETHING IN BETWEEN?

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Keywords: alternative treatments, off-label treatments, patient choice

Only a small percentage of patients with ALS enroll in research studies. At the same time, many patients with ALS will consider alternative and off-label treatments (AOTs) they read about on the Internet. These surprising decisions can have important consequences. Slow enrollment means studies take longer, cost more, may be terminated without a conclusion, and may not be generalizable even when they are completed. Pursuit of AOTs can result in financial, psychological, physical and scientific harms. Survey data suggest that patients may make decisions about research and AOTs using information that is scant, flawed or even inaccurate. There thus exists an opportunity for health care professionals (HCPs) to assist patients with these important decisions.

Using a case-based format, this presentation will compare and contrast four classic models by which HCPs might assist patient choices toward research studies and/or AOTs: paternalistic, informative, interpretive, and deliberative (1). We will show that these are distinguished by how they define patient values, by their concept of patient autonomy, by how they view HCP obligations, and by the goals they set for the HCP-patient interaction. We will describe the real-world examples of each model being employed in ALS. These will include: face-to-face clinic visits, "compassionate use" programs, ALSUntangled and the ALS Clinical Research Learning Institute (ALS-CRLI). While each has its strengths and weaknesses, we will argue for the deliberative model being employed by ALS-CRLI as being optimal.

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C21 CHOICES AND CONTROL - INVESTIGATING THE NEEDS, THOUGHTS AND WISHES OF PEOPLE WITH MOTOR NEURONE DISEASE IN THE AREA OF DEATH, DYING AND END-OF-LIFE DECISION MAKING

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Keywords: death, suicide, end-of-life

Background and objectives: Picker Institute Europe conducted a qualitative study investigating the views of people with ALS/MND in the areas of death, dying and end-of-life decision making. The objectives of the cross-sectional qualitative study were to extend understanding concerning death, dying and end of life decision-making from the perspective of people with ALS/MND, in order to inform future service development and support activities by the study sponsor (MND Association).

Methods: Study design was informed by preliminary focus groups, comprising 'front-line' staff and volunteers from the MND Association. Participants with ALS/MND for the subsequent 'opt-in' qualitative study phase were recruited from across these regions via MND Association publicity. Thirty-four semi-structured interviews (21 male, 13 female) were conducted. A choice of interview methods was offered: face-to-face, by telephone, and through email or other communication device. This mix of approaches aimed to ensure geographical coverage and inclusivity. The sample included a broad range in terms of length of time since diagnosis (< 1yr -> 6 yrs). However, some of the more recently diagnosed interviewees reported symptoms for several years (pre-diagnosis), indicating that the sample was possibly skewed towards those with more slowly progressive disease. After 34 interviews 'saturation' point had been reached, with themes and issues confirmed without new material arising.

Results: Three key themes emerged relating to end-of-life concerns:

Discussing death and dying – although difficult, talking about end-of-life issues can be helpful. However, end-of-life topics may be taboo even for those working with terminally ill people. Some interviewees felt the Patient Association had a role in changing attitudes, encouraging people to speak freely and providing information on all aspects of death and dying.

Self-determination – many participants equated dignity in dying with having control and choices and being able to make their own decisions at the end-of-life. Defining what was an

acceptable quality of life and deciding when and where they wanted to die was important. Some wanted assisted dying as a possible option at the end-of-life, including a number who stated they might not choose it for themselves.

Having a voice – many participants said that a public debate on end-of-life issues, and in particular, assisted dying was needed. There was concern that healthy people were making policy decisions and legislation on these matters without listening to those affected by conditions like MND.

Discussion and conclusions: There is a need to increase the provision of clear, up to date information on end-of-life decision-making and choices (including possibly legal summaries on assisted dying) whilst continuing to improve access to MND specialist medical/palliative care and training of health professionals. Improved awareness and training of health professionals and Patient Association staff/volunteers in patient attitudes to death and dying is also required, in order to facilitate open discussion of options and choices for end-of-life, in a manner that addresses both individual diversity and the stages of disease progression.

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C22 WIND OF CHANGE – WELL-BEING AND DECISIONS IN THE COURSE OF ALS

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Keywords: quality of life, depression, decisions

Background: Decisions to prolong or shorten life in fatal diseases are highly unknown. Furthermore, it is not clear how a patient adapts his decisions in the course of the disease. It was the aim of the study to determine 1) decisions of patients towards life prolonging treatments 2) course of change throughout 1.5 years 3) determinants of decisions.

Material and methods: Decision process of 94 ALS patients in advanced stages of the disease (T1 mean ALS-FRS 27.86 ± 11.2) was measured longitudinally with a semi-structured interview four times in the course of 1.5 years. They were interviewed on quality of life, attitudes to treatment and preferred treatment every six months for 1.5 years (T1 to T4).

Results: Indicators of high quality of life (T1 mean SeiqoL = 71.7, T4 = 66.4; F = 0.05, p = 0.08) and global quality of life (T1 mean ACSA = 0.01 T4 = 0.6; F = 0.28 p = 0.06) and low depression rate (T1 mean ADSK = 8.6, T4 = 9.2; F = 0.04 p = 0.06) were stable throughout the study. In this cohort, the wish for hastened death was extremely low (T1 mean SAHD = 4.9) and decreased during the first six months (T2 mean SAHD = 3.8, F = 6.72 p = 0.01). It remained low throughout the study (T4 SAHD = 2.9, F = 3.5 p = 0.01) despite the fact that physical function declined (T4 mean ALS-FRS = 20.27; F = 3.04 p = 0.09). There was a positive correlation of the feeling to be a burden and the wish for hastened death (r = 0.46 p = 0.03) and depression (r = 0.25 p = 0.03), respectively. Initially, up to half of the patients (52%) had a positive mental attitude towards life-sustaining treatments, about one third were undecided (35%). Of the 48% with negative or undecided mental attitudes 10% changed towards acceptance in the course of the study.

Conclusion: In conclusion, ALS patients in Germany reported a high quality of life and a low desire for hastened death. The fear to be a burden for others was associated with a high wish for hastened death and low well-being. Positive attitudes regarding life-sustaining treatments were predominant either initially or during the course of the disease. Consistent with other studies, our data provide no evidence for a general end-of-life-oriented despair in ALS and instead a positive attitude towards life-prolonging therapeutic treatments.

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C23 ASSESSMENT OF END OF LIFE SETTING IN ALS PATIENTS ATTENDING A MULTI-DISCIPLINARY CLINIC

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Keywords: end of life, medical power of attorney, multi-disciplinary clinic program

Background: End of life planning in patients with ALS (PALS) is important even in its early stages so that their autonomy, especially in placement at end stage, is optimized. In our multi-disciplinary clinic (MDC), we have an ongoing dialogue with each PALS and their families to ascertain where they wish to be at end stage. Ideally, all elect to remain at home, with few needing long term care facility or inpatient hospice.

Objectives: To determine how many of the PALS in our MDC are able to make and preserve their choice at end stage of disease and what factors might relate to deaths in acute hospitalization at end stage.

Methods: The ALS Association Wisconsin Chapter PALS database was reviewed for deaths during a 20 month period (November 2009 - July 2011): 54 attended the ALS MDC. Information was obtained about their end of life, including date/location (home, residential facility, or acute hospitalization) of death, existence of medical power of attorney (MPOA), and disease duration.

Results: 3 of the 51 deceased PALS were excluded for lack of adequate information, leaving a total of 48 PALS for study. We divided these into 2 groups. In group one (41 PALS), 34(71%) deceased at home, 2(4%) deceased in an inpatient hospice, and 5(10%) deceased in a long term care facility. 32 (78%) had MPOA in place at least one month prior to death. 26 (63%) PALS in this group were in hospice. 7 PALS in group 2 (15%) deceased during an acute hospitalization (mean length of stay 7 days, range 2–15 days). 4 had no MPOA, one had MPOA completed the day before admission, and 2 had MPOA in place prior to their disease but had not updated it after diagnosis of ALS. Only one PALS in this group was in hospice. Mean disease duration in group 1 was 35 months (range 4–216 months) compared to group 2 (43 months, range 15–64 months).

Discussion: Our MD ALS Program integrates palliative care throughout a PALS' management at all stages. The autonomy of each PALS is a major part of the MD team's efforts to provide education and resources to them and their families so that they make informed choices and avoid unnecessary interventions and hospitalizations, particularly at end stage. Repeated discussion helps prepare PALS and their families for end stage disease, which facilitates transition to hospice programs.

Conclusions: This study emphasizes the importance of integrating palliative care early in the management of ALS, including the use of the MPOA to facilitate these decisions. PALS who deferred or did not update MPOAs and were not under hospice care were more likely to decease in hospital.

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C24 ALS PATIENT REPORTS OF END OF LIFE PREFERENCES

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Keywords: end of life, advance care planning, evidence based practice

Background: There are currently no ALS specific guidelines for end of life (EOL) care. Traditional advance directives often are not adequate to help ALS patients plan for treatment options or for their clinicians to make care decisions consistent with patients' goals.

Objective: To gather information from patients with ALS about preferences for medical treatment at EOL.

Methods: A questionnaire was developed to solicit information on patient EOL preferences, and was posted as an on-line survey. Patients receiving care at the Penn State Hershey ALS Center were notified of the study and offered the opportunity to complete the survey. Responses were analyzed using descriptive statistics and frequency counts. The study was approved by the Penn State Hershey Medical Center Institutional Review Board.

Results: 40 patients completed the questionnaire. The sample included patients seen in the clinic less than 6 months (7.5%), between 6–12 months (22.5%), between 1–2 years (22.5%) and longer than 2 years (47.5%) Mean ALSFRSR was 17.3, SD 8.5. Two-thirds had thought "a fair amount" to "a great deal" about EOL preferences, while one-third reported thinking "not at all" to "a little bit" about these wishes. 82.1% of patients reported preparing an advance directive or living will, and of those, 90.6% reported satisfaction with their document. The primary reason for not completing an advance care planning document was "my loved ones know my wishes and they did not see the need to have it in writing" (42.0%) followed by "I'm not ready to think about this issue yet" (28.6%) and "I'm not sure why I haven't done one yet" (14.3%). When questioned about who should begin conversations about EOL care, top responses included the neurologist (60.5%), the patient (55.3%) and a family member or loved one (52.6%). A smaller percentage reported that the family doctor should begin EOL conversations (21.1%). Respondents most commonly thought that the time to initiate EOL discussion was when ALS symptoms change (79.5%), when the patients' wishes for EOL care change (59%), and when ALS team members think it is time to discuss EOL (43.6%); less frequent responses included "at the first ALS clinic visit" (17.9%) and "at every ALS clinic visit" (12.8%).

Discussion and conclusions: Patients who are followed in an ALS clinic often have thought about EOL care. They believe that discussions about EOL care should be initiated when ALS symptoms change. They frequently look to their health care team to determine when initiate such discussions, and to their ALS physician to do so. This information is being used as the basis for an evidence-based project on ALS end of life care.

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SESSION 4A GENETICS & GENOMICS

C25 MOTOR NEURON INVOLVEMENT IN MULTI-SYSTEM PROTEINOPATHY: IMPLICATIONS FOR ALS/MND

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Keywords: multisystem proteinopathy, inclusion body myopathy, frontotemporal dementia

Background: Inclusion body myopathy with Paget's disease and frontotemporal dementia (IBMPFD) was initially described as an autosomal dominant, multi-system degenerative disease with incomplete penetrance of each characteristic feature. Early reports described mutations in the valosin-containing protein (VCP) gene as the cause of IBMPFD, and the disabling weakness of this disorder has historically been attributed to muscle disease. We recently identified mutations in the VCP gene in several individuals with familial ALS, and suggested that the phenotypic spectrum of IBMPFD extends to include motor neuron disease.

Objectives: Ascertain the frequency with which degeneration or dysfunction of motor neurons contributes to weakness in patients with IBMPFD.

Methods: To date, 17 study participants from 8 families with neuromuscular weakness, Paget's disease, and/or FTD have been characterized using neurological and electromyographic examinations as well as genetic analysis.

Results: Weakness (median age of onset 38, range 25–52) was the most common clinical manifestation (present in 15 patients), with physical signs of upper motor neuron dysfunction in four patients. EMG was abnormal in all 17 patients, showing purely neurogenic changes in $n = 5$, purely myopathic features in $n = 6$, and a mixture of neurogenic and myopathic changes in the remaining $n = 6$. Mutations in the VCP gene (R155H, R159G, R155C) were identified in six families, and an unpublished new gene was identified in another family. The genetic cause in the eighth family has not yet been identified. There were no clear genotype-phenotype correlations.

Discussion and conclusion: The heterogeneity of IBMPFD has grown to encompass at least four disease genes and the phenotypic spectrum extends beyond IBM, Paget's Disease and FTD. Importantly, weakness, which is the most common and disabling phenotypic feature, may be caused by intrinsic muscle dysfunction, motor neuron disease, or a combination of the two. The acronym IBMPFD is therefore insufficient to explain disorders due to mutations in VCP or other recently identified IBMPFD-associated genes. Instead, we favor the descriptor Multi-System Proteinopathy (MSP), using MSP1 for a disease associated with VCP mutations and MSP2, MSP3, etc. for diseases with mutations in other known genes. The term 'MSP' encompasses not only the extended clinical phenotype, but also the previously described prominent pathologic feature of protein aggregation in affected tissues. The genetic defects in MSP implicate a range of biological mechanisms including RNA processing and protein homeostasis. These mechanisms may also be relevant to the pathobiology of more common motor neuron degenerative diseases such as ALS – and provide an additional link between ALS and FTD.

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C26 SOMATIC HETEROGENEITY OF THE GGGGCC HEXANUCLEOTIDE REPEAT IN C9ORF72 EXPANDED REPEAT CARRIERS

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Keywords: C9ORF72, southern blot, repeat length

Background: Linkage analysis in autosomal-dominant families in which affected members develop amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) or both, and where the pathology is TDP43-positive, have long suggested a major locus for FTD/ALS on chromosome 9p21. Last year, we identified a GGGGCC hexanucleotide expanded repeat in the non-coding region of chromosome 9 open reading frame 72 (*C9ORF72)* as the mutation anomaly responsible for disease in these families and the most common cause of ALS and FTD to date. Using southern blot analysis with DNA extracted from lymphoblast cell lines of *C9ORF72* mutation carriers, we showed variable GGGGCC repeat lengths (700–1600 repeats); however, the repeat length in affected brain tissue and non-affected peripheral tissue samples from *C9ORF72* mutation carriers has not been systematically studied.

Objectives: To determine the GGGGCC repeat size and degree of heterogeneity in DNA samples from different brain regions and non-affected peripheral tissues in *C9ORF72* mutation carriers.

Methods: We studied three ALS patients with *C9ORF72* expanded repeats ascertained at the ALS Center at Mayo Clinic Florida with full autopsy available at the Mayo Clinic

Florida Brain Bank. Genomic DNA (gDNA) was extracted from blood, spleen, heart, muscle, liver and different brain regions (frontal cortex, temporal cortex, parietal cortex, occipital cortex and cerebellum) and used for southern blot analysis.

Results: The *C9ORF72* mutation carriers presented clinical features of classical ALS with the exception of one patient diagnosed with progressive muscular atrophy (PMA) without upper motor neuron signs. TDP-43-positive pathology was confirmed in all patients. Post-mortem examination showed classical ALS pathology in two cases and FTLD-MND with predominantly lower motor pathology in the PMA patient. Southern blot analysis using DNA extracted from several brain regions, peripheral tissues and blood confirmed the presence of an expanded allele with a smear of high molecular weight bands in all cases, suggesting somatic instability of the expanded repeat. Direct repeat size comparison of gDNA from blood and cerebellum showed no significant difference in size in two cases, whereas the third case diagnosed with PMA showed only 80–100 repeats in blood and >1000 repeats in the cerebellum. We further detected variable degrees of somatic heterogeneity of repeat size in the expanded alleles within and across tissues in all affected individuals. The longest repeat lengths were generally observed in the brain.

Discussion: The repeat length in *C9ORF72* mutation carriers is highly variable across tissues as a result of somatic instability. The exact mechanism for the instability is poorly understood; however, several factors, including DNA replication, repair, recombination and transcription, may be involved. The discrepancy of *C9ORF72* repeat length between blood and brain tissue could have implications for diagnostic testing and should be considered when performing correlative studies of repeat size with clinical and pathological endophenotypes.

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C27 EVIDENCE FOR AN OLIGOGENIC BASIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oligogenetic inheritance, C9ORF72, TARDBP

Background: In pedigrees affected by its familial form of ALS, incomplete penetrance is often observed as well as high phenotypic variability. We hypothesized that this could be caused by complex inheritance of multiple risk variants in multiple genes.

Objective: We screened 111 FALS patients from 97 families, and large cohorts of SALS patients (n > 1,000) and control subjects (n > 1,000) for mutations in *TARDBP*, *FUS/TLS*, *SOD1*, *ANG*, and *C9ORFf72* in search of evidence for oligogenetic inheritance.

Results: Mutations were identified in 48% of FALS families, 8% of SALS patients, and 0.5% of control subjects. In five of the FALS families, we identified multiple mutations in multiple ALS-associated genes. We detected *FUS/TLS* and *TARDBP* mutations in combination with *ANG* mutations, and *C9ORF72* repeat expansions with *TARDBP*, *SOD1*, and *FUS/TLS* mutations. Statistical analysis demonstrated that the presence of multiple mutations in FALS is in excess of what is to be expected by chance ($p = 1.77 \times 10^{-7}$). The most compelling evidence for an oligogenic basis was found in individuals with a p.N352S mutation in *TARDBP*, detected in five FALS families and three apparently SALS patients. Genealogical and haplotype analyses revealed that these individuals shared a common ancestor. We obtained DNA of 14 patients with this *TARDBP* mutation, 50% of whom had an additional mutation (*ANG*, *C9ORF72* or homozygous *TARDBP*).

Discussion and conclusions: We provide strong evidence for an oligogenic etiology of ALS. This may have important implications for the interpretation of whole exome/genome experiments designed to identify new ALS-associated genes, and for genetic counselling, especially of unaffected family members.

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C28 IDENTIFICATION OF NOVEL ALS GENES USING LINKAGE ANALYSIS AND EXOME SEQUENCING

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Keywords: exome, linkage, bioinformatics

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes the progressive degeneration of motor neurons. Familial ALS accounts for approximately 10% of ALS cases, with the remainder being sporadic. ALS is genetically heterogeneous. To date, known genes account for ~55% of familial cases.

Objectives: We aim to investigate known ALS genes and identify new ALS genes in a large cohort of Australian ALS families (n = 187) using a combination of sequencing, traditional genetic linkage approaches and next-generation

sequencing strategies. We also aim to implement a user-friendly pipeline for an analysis of exome sequencing data.

Methods: A large cohort of Australian ALS families (n = 187) has been recruited. We are using a combination of traditional genetic linkage approaches, together with next-generation sequencing strategies, to search for new ALS genes among families that are negative for mutations in all known ALS genes. Known ALS genes have been the ongoing subject of mutation analysis among these cohorts.

Results: We analysed 187 ALS families for mutations in known ALS genes and determined that they account for 57.2% of Australian ALS families and comprise *SOD1* (13.9%), *FUS* (2.7%), *TARDBP* (2.1%), *UBQLN2* (1.1%) and *C9ORF72* (38.0%) mutations. In order to identify new loci for familial ALS, an 8cM genome-wide microsatellite linkage scan was performed on 51 individuals (affected, unaffected and obligate carriers) from two large informative families negative for all known ALS genes. Previous linkage analysis was performed on one of these families using a 10K Affymetrix SNP chip. Subsequent analyses have yielded significant and suggestive linkage to several chromosomal regions. In order to reduce sequencing burden when new ALS genes are identified, a proband from each of our remaining 79 families was subjected to exome capture and sequencing (Agilent capture-SOLiD4 sequencing or Illumina TruSeq capture-HighSeq2000 sequencing). Bioinformatic analysis has required the development of a user-friendly pipeline. We have two novel candidate genes that are currently being validated.

Discussion and conclusions: The genetic defects are yet to be identified among 42.2% of ALS families (79/187 families) within our cohort. The chromosomal regions implicated from our genome-wide linkage scans do not overlap previously identified loci, implicating substantial genetic heterogeneity. Linkage analysis, in combination with exome capture and sequencing, has allowed us a greater opportunity to identify novel ALS genes. Two candidate genes are currently being validated in extended patient and control cohorts, patient tissues and functional studies. The identification of these novel ALS genes will give insights into the biological basis of both familial and sporadic motor neuron degeneration, allow development of new disease models and provide new targets for therapeutic development.

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C29 RESIDUAL ASSOCIATION OF CHROMOSOME 9P21 SNPS WITH ALS AFTER EXCLUSION OF C9ORF72 MUTATED CASES

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Keywords: genetics, 9p21, statistics

Pathological expansion of a hexanucleotide repeat in an intron of the C9ORF72 gene is a cause of about 10% of all ALS, and was identified through a series of linkage and association analyses. The risk SNPs and haplotype that tag the mutation are frequent in the general population. It is possible that the mutation is not the only disease causing variation in this region. For example, in Parkinson's disease, a situation exists in which linkage is seen in families to the same genomic region as those with no family history, but is caused by two different genetic lesions; one a Mendelian, high penetrance mutation, the other, common variation at a SNP.

Aim: To identify whether the 9p21 risk haplotype still associates with the disease when the mutation has been accounted for.

To remove the ALS cases with the mutation from our dataset to elucidate other SNPs genome-wide that associate with ALS.

Method: We screened case samples previously analysed in a genome-wide association study (GWAS) for the pathological expansion of C9ORF72. We stratified further analyses by the presence or absence of the expansion, examining the locus for residual association, and analysing for genome-wide association.

Results: There were 599 case samples and 4142 controls. A total of 39 of the cases were expanded. Controlling for the presence of the expansion, there was residual association at chromosome 9p21, (rs3849942 p-value = NNN). Genome-wide, no new loci were identified in the more homogeneous sample. A previously identified ALS-associated haplotype did not show association once the expanded cases were accounted for.

Conclusions: There may be further disease-causing variation at the chromosome 9 locus, possibly resulting in synthetic association.

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C30 TOTAL TRANSCRIPTOME SEQUENCING ANALYSES IN BRAIN TISSUE OF C9ORF72 EXPANDED REPEAT CARRIERS

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Keywords: C9ORF72, RNA sequencing, expression

Background: We recently identified expanded GGGGCC repeats in the non-coding region of the chromosome 9 open reading frame 72 (*C9ORF72*) gene as the long sought-after cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) on chromosome 9p. In line with studies in other non-coding repeat expansion disorders, we showed that the repeat expansion leads to the formation of nuclear RNA foci, suggesting a possible toxic RNA gain-of-function disease mechanism.

Objective: To identify gene expression and alternative splicing changes resulting from GGGGCC repeat expansions in *C9ORF72* using transcriptome sequencing.

Methods: We performed total RNA sequencing (RNAseq) using RNA extracted from frontal cortex from 16 FTLD patients with TDP43 pathology from the Mayo Clinic brain bank. Eight patients carried expanded *C9ORF72* repeats, while normal repeat lengths were present in the other eight. Patient groups were matched for age at death, sex and brain weight, and all samples had RNA integrity numbers >8. RNAseq was performed using an Illumina HiSeq2000, one sample per lane. The Illumina standard processing pipeline v1.5 was employed for processing raw images to make base calls and generate sequence reads, which were aligned to human genome assembly 19 and our in-house exon junction database. Aligned sequence tags were counted for each annotated gene/exon using our in-house RNA-seq pipeline SnowShoes-EX. A total of 22480 genes were annotated using RefSeq RNA database and raw read counts for genes were generated for downstream analyses.

Results: Differential gene expression analysis was performed using DESEQ, showing 367 genes as differentially expressed between groups (adjusted $p<0.01$). Pathway and functional enrichment analysis using Ingenuity Pathway Analysis tool showed that 40 of these genes were annotated to neurodegenerative disorders (overlap $p=3.25E-09$). Significant networks identified were related to neurological disease, cell-to-cell signaling and interaction, and inflammatory response. Interestingly, GABA receptor signaling and ALS signaling were among the four most enriched canonical pathways in *C9ORF72* mutation carriers compared to non-carriers. The identification of ALS signaling is exciting, given that *C9ORF72* expansions can cause both FTLD and ALS, but only FTLD brains were included in this study. To determine whether relative expression levels of transcripts were changed, we used Partek Genomic Suite. Twenty-seven genes were estimated to be alternatively spliced between groups ($p<0.001$), with diacylglycerol kinase, zeta (*DGKZ*) and glutamate receptor, ionotropic, N-methyl D-aspartate 1 (*GRIN1*) as the most significantly alternatively spliced genes. To detect differential exon usage across the two groups, we further employed DEXSEQ which identified additional genes with differentially expressed exons ($p<0.001$), including ubiquitin-conjugating enzyme E2A (*UBE2A*) and ubiquitin interaction motif-containing protein 1 (*UIMC1*).

Discussion: RNAseq shows differentially expressed genes and alternative splicing changes in patients carrying *C9ORF72* repeat expansions. The specific targets identified in this study need confirmation in additional patient series and tissues.

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SESSION 4B CARER & FAMILY SUPPORT

C31 HOW CAN WE CARE FOR THE CARERS?

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Keywords: carer, stress, loss

Family carers are vital to the welfare of those with MND, yet being a carer for someone with progressive disability is known to be hard work and can take a toll on the carer's mental and physical health. This talk will consider how themes arising from research can inform practice; in particular, the ways carers can be supported and enabled to sustain their caring role without damaging their own well-being. A number of key themes from recent caregiver research with MND and other conditions will be presented and explored, and implications drawn out for carers and those who work with them.

This research, for example, demonstrates the power of the mind, in that the stress of providing care has a stronger link with the way the carer 'makes meaning' of their situation than with the type or degree of support. Therefore considering how carers can think of their situation differently becomes relevant.

Another thread of recent research focuses on the relationship within which caring takes place, showing how its history intersects with the shift in workload and power that accompanies illness. Thus, 'soft' issues such as how decisions are negotiated, within a couple and with services, become as important to address as those around instrumental care.

Carer research has only more recently recognised that grief processes can be as powerful as stress processes in caregiving, as carers witness changes in their relative. Research into dealing with 'anticipatory grief' and 'chronic sorrow', therefore, becomes relevant here to inform whether being in touch with feelings is helpful during the care trajectory.

Evidence-based psychotherapeutic developments also have potential for transfer to the MND setting, with self-compassion (kindness towards, and acceptance of, oneself) providing one promising approach that appears to be protective against depression in demanding situations.

Overall then, research in the MND area and further afield has much food for thought to offer as we consider how to support carers. This presentation will provide a synopsis of key strands of research with ideas about how it might translate into practice.

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C32 LIVING WITH MND: A CARER PERSPECTIVE

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Keywords: carer burden, qualitative, quality of life

Background: A diagnosis of motor neurone disease (MND) has a substantial impact within a family. The effect on carer-partners is increasingly recognised, particularly in terms of anxiety, depression and reduced quality of life. Attention to informal carer well-being is important not only for the carer but also for the patient because of the significant association observed between carer well-being and patient well-being.

Objectives: The objectives of this study were; i) To describe the experiences of informal carers of patients with MND over the course of the disease and ii) To make recommendations regarding how support for carers may be enhanced.

Methods: The study used a longitudinal mixed-method approach including interviews and questionnaires with MND patients and their carers over the course of the disease. The work reported in this paper focuses on the qualitative interviews carried out with carers, together with data from the SF36 carer measures and Carer Strain Index.

Results: Seventeen carers took part in the study. A key theme in the data was the importance of maintaining patient independence and using strategies to preserve this as long as possible. A second theme identified was the considerable physical effort of the carer's role. Related to this was the change in the role of the partner from an informal carer to a role almost indistinguishable from a formal carer. Carers described how, while having time away could be beneficial, that feelings of worry or guilt impacted on their ability to leave the patient. Coping strategies, such as focusing on each day rather than thinking about the future, and trying to maintain a positive or cheerful outlook, were described. Key aspects of service provision highlighted were: a reluctance to use professional carers; the intrusion of services into life, particularly soon after diagnosis; and issues regarding timing of equipment provision. Data from the quantitative measures echoed the physical burden on carers with the SF36 Physical Component Summary score considerably below that of the Mental Health Component Summary score (mean 0.5 (SD 15.4) vs mean 14.6 (SD 14.3)) at baseline and at all following time points.

Discussion: The study highlights the need for not only the emotional elements, but also the physical impact of caring for a patient with MND to be recognised. It suggests the importance of multiple services co-ordinating their input, particularly in the early stages following diagnosis, in order to provide

timely support whilst avoiding overwhelming the patient and the carer. The data describe the considerable challenge for many carers in having time away from the patient and accepting professional services which need to be overcome if respite or care services are to be taken up. Finally, the study highlights the importance of providing equipment at the optimum time.

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C33 INTERPERSONAL RELATIONSHIPS AND PURPOSE IN LIFE ARE PREDICTORS OF SURVIVAL IN ALS

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Keywords: purpose in life, interpersonal relationships, survival

Background: Previous studies have suggested that psychological well-being may predict physical status/survival in people with ALS. Studies have also indicated that executive dysfunction may be a prognostic indicator. Little information within ALS is available concerning the impact of social relationships on survival.

Objectives: Within the context of a study of decision-making in people with ALS, we investigated the prognostic value of psychological well-being (reflected by purpose in life- PIL), cognitive function, symptom severity, body mass index (BMI) and whether the person with ALS could be conceptualised as being or having been in a supportive relationship.

Methods: Measures of purpose in life (PIL Scale), cognitive function (ACE-R), symptom severity (ALSFRS-R), BMI and time from symptom onset were obtained in 78 people with ALS recruited to a population-based study. These variables, together with the classification of whether the person was married/living with a partner/widowed vs single/divorced), were used to predict survival during the study.

Results: Of our sample, 58 people were married, four were in a stable partnership and six had been widowed; in addition, three were single and seven were divorced/separated. In the final model, using a Cox survival analysis, survival was independently predicted by diagnostic delay ($B = -0.077$, $se = 0.031$, $p = 0.012$) region of disease onset ($B = 1.688$, $se = 0.023$, $p = 0.012$) and ALSFRS-R Total ($B = -0.120$ $se = 0.054$, $p = 0.026$). Age, gender, body mass index and total ACE-R scores did not predict survival. However, PIL scores at baseline modestly but significantly predicted survival ($B = -0.028$, $se = 0.013$, $p = 0.031$) as did the nature of the relationship in which the person had been involved. Being married/living with a partner/widowed predicted a longer survival time as did being single/divorced ($B = 1.978$, $se = 0.680$, $p = 0.004$).

Discussion and conclusions: Our results confirmed a number of accepted prognostic factors for survival. We also found evidence, supporting earlier studies, suggesting that psychological well-being may be an important predictor of survival in ALS. Higher PIL could indicate that the person experiences a greater sense of meaning in life; a lack of purpose in life might influence a person's ability to confront challenging life situations. It is possible that this may influence how people deal with the challenges of ALS. Additionally, we found an independent, further predictor related to the nature of the intimate relationships in which participants had been involved. It is unlikely that this simply reflects the presence of a carer, since there was no predictive difference between those currently living with a partner or not when the widowed participants were reclassified along with the single/divorced people. Further research needs to investigate the impact of the nature of the significant relationships in dealing with illness and how these might relate to other disease phenotypes and traits which may influence survival.

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C34 DECISION-MAKING ABOUT REPRODUCTIVE CHOICES AMONG INDIVIDUALS AT RISK FOR FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS IN FAMILIES WITH A KNOWN GENETIC MUTATION

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Keywords: familial ALS, reproductive decision-making, genetic testing

Background: No research has focused on the reproductive decision-making process in individuals at risk for familial amyotrophic lateral sclerosis (FALS).

Objectives: This qualitative study aimed to explore the process of reproductive decision-making in individuals at 50% risk for FALS in families with known genetic mutations.

Methods: Utilizing a semi-structured interview, we spoke with ten at-risk individuals recruited from the Northwestern Neurologic Diseases Registry. Participants had a first-degree relative affected with FALS, made reproductive decisions in the past 30 years and did not know their genetic status when they made reproductive decisions. Using qualitative content analysis, we delineated themes that emerged in individuals who chose to have children and those who chose not to have children, and themes that described the process as a whole.

Results: Those who chose to have children believed that regardless of disease onset, life can be productive. They compared ALS relatively favorably with other diseases, had always planned on having children and hoped a cure would be found in the near future. Individuals who chose not to have children tended to have an extensive experience with ALS and associated caretaking, saw ALS as a tragedy that is inevitable for themselves or their family members and avoided serious relationships. In conversation with their partners, all individuals considered other reproductive options beyond the one they ultimately chose. Conversations about reproductive decisions and risk for ALS varied in length, and often strengthened relationships. A primary concern for participants was children experiencing the death of a parent at a young age. No participant regretted the decision they made. Individuals were motivated to pursue predictive genetic testing to help others

in their family and because they currently saw symptoms i n themselves that produced uncomfortable levels of anxiety.

Discussion and conclusions: The results of this exploratory study show that the decision-making process is complex. Our results can guide future research about reproductive decision-making and predictive testing in individuals at risk for FALS, as well as provide direction for genetic counsellors and other healthcare professionals when working with individuals during the family planning process and prior to predictive genetic testing.

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C35 SPECIFIC PHOBIA OF AMYOTROPHIC LATERAL SCLEROSIS (ALS PHOBIA)

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Keywords: specific phobia, psychotherapy, affective disorders

Background: Specific phobias comprise 12.5% of affective disorders (1).

Objectives: To our knowledge, the phobia of ALS has not been described in periodicals previously. Only information about affective disorders in people whose relatives were diagnosed with familial ALS was found.

Methods: In 2006–2012, we examined 21 patients (11 males and 10 females within an age range of 28–72 years). Among them were three ALS patients' relatives, four neurologists and 14 patients who received information about ALS from other sources. Eight patients had another disease, five had premorbid psychiatric problems (23%) and eight were healthy. Patients underwent needle EMG at baseline and completed Hamilton Depression Score (HDS) at baseline, 3 and 6 months after initial visit and treatment. The compliance rate for HDS was 81%, 61% and 52%, respectively. A statistical analysis was performed by Mann–Whitney criterion and Spearman correlation.

Results: The clinical picture of ALS phobia was represented by an obsessive feeling of generalized muscle twitching, an obsessive self-analysis of symptoms and intention to perform new examinations, anxiety, depression and insomnia. Ten patients had mild, six had moderate and five had a severe phobia (6 ± 1, 14 ± 3 and 23 ± 2 degrees of HDS, respectively: $p < 0.05$ for each comparison). The duration of the phobia was significantly higher in patients with moderate and severe phobia than in mild cases (1.5 ± 0.6 and 5 ± 1.1 months, respectively, $p < 0.05$). The severity of ALS phobia correlated with its duration ($R = -0.5$; $p = 0.004$). The primary character of phobia was established on the basis of regression of signs (monitored by HDS) after psychotherapy and pharmacotherapy in 52.4% of patients (17 ± 4 and 3 ± 1 degrees of HDS before and after 3 months of treatment, $p < 0.05$). In mild ALS phobia, we prescribed a timoleptic, an antidepressant and a hypnotic, in moderate phobia – an atypical neuroleptic was added, in severe phobia – additional antidepressant and atypical neuroleptic were added. On a final telephone interview in 2012, none of these patients developed ALS.

Discussion: We propose that we have not found previous descriptions of this phobia in the literature due to higher prevalence of affective disorders in Russia compared with Europe and the USA (1–3). It is feasible to take into account that such a phobia may occur while presenting information about ALS to healthy people.

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SESSION 7A MODELLING ALS

C36 MODELLING ALS WITH EMBRYONIC STEM CELL-DERIVED MOTOR NEURONS

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Keywords: embryonic stem cells, transcriptional programming, motor neuron diversity

Despite recent progress in creating cell models of disease, it has proven difficult to mimic *in vitro* the selective degeneration of vulnerable motor neuron populations in ALS patients. Embryonic stem cells can be directed to differentiate with high efficiency into motor neurons. We used mouse ES-derived motor neurons to screen for stressors that elicit selective degeneration of ALS motor neurons carrying the SOD1-G93A transgene. The effect of identified stressors was then tested in human control and patient iPS-derived motor neurons. The ES/iPS-MNs generated by standard methods are cervical in character. To better model the disease, it may, therefore, be important to program them into ALS-vulnerable and ALS-resistant populations, respectively. We demonstrate that differentiating stem cells can be precisely programmed to distinct motor neuron subtypes by inducible expression of transcription factors. We plan to use these populations to better analyse the cellular and molecular mechanisms of selective neurodegeneration in ALS and to screen for neuroprotective agents.

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C37 EMBRYONIC STEM CELL-DERIVED MOTOR NEURONS GENERATED FROM TDP-43 (A315T) MICE DEVELOP ALS-LIKE PATHOLOGY

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Keywords: embryonic stem cells, TDP43, motor neurons

Background: Recent findings in ALS research have highlighted the possible role of TDP43 as a central player in several neurodegenerative diseases. TDP43 is known to accumulate in ubiquitinated inclusions of affected neurons, suggesting that the loss of normal TDP43 function as a nuclear protein or, alternatively, a gain of a toxic function by TDP43 aggregates may play a critical role in the pathogenesis. The mechanisms of TDP43 functions are currently being investigated in yeast and transformed cell lines; however, the role of these interactions in motor neurons is not entirely known.

Objectives: 1) To determine the timecourse of disease pathology and define disease-specific changes in motor neuron gene expression. 2) To investigate the cell-autonomous and non-cell autonomous effects that lead to neurodegeneration in TDP43(A315T) ES cell-derived motor neurons. 3) To do a biochemical analysis of mTOR signaling pathway that likely plays a key role in the mislocalization of TDP43 aggregates in TDP43(A315T) ES cell-derived motor neurons. 4) To investigate whether rapamycin rescues TDP43 mislocalization and subsequent gene expression phenotypes in TDP43(A315T) ES cell-derived motor neurons.

Methods: ES cell-derived motor neurons were generated from transgenic mice expressing a mutant human TDP43(A315T); Hb9: GFP transgene. Green fluorescent protein (GFP) expression is used to identify putative motor neurons, because GFP is expressed under the control of the Hb9 promoter, a gene expressed by all post-mitotic motor neurons.

Results: Preliminary results indicate that ES cell-derived motor neurons from transgenic TDP43(A315T) mice display ALS-specific motor neuron phenotypes in culture, including development of ubiquitinated cytoplasmic TDP43 aggregates and $>80\%$ cell death by 28 days post differentiation. In addition, ~30% of ubiquitinated aggregates in ES cell-derived motor neurons generated from TDP43(A315T) mice colocalize with FUS. Furthermore, while there are cell autonomous effects, preliminary findings using FACS sorted control GFP + motor neurons plated with mutant TDP43 non-GFP cells indicate that non-cell autonomous disease effects can be observed with $>80\%$ cell death of control motor neurons in the presence of mutant non-GFP cells. This suggests that signals are transmitted by the mutant non-GFP cells (non-motor neuron fraction) either through cell-cell contact or diffusion of toxic factors. Finally, preliminary results also show a decrease in TDP43 cytosolic aggregates and an increase in the survival of TDP43(A315T) ES cell-derived motor neurons when treated with Rapamycin.

Conclusions: Motor neurons derived from TDP43(A315T) ES cells recapitulate ALS disease phenotypes similar to human ALS pathology.

Discussion: These studies will serve to establish ES cell-derived motor neurons as pre-clinical *in vitro* models for investigating both sporadic and familial ALS disease mechanisms. Finally, the discovery of factor(s) responsible for motor neuron death can be used as biomarkers for early diagnosis of ALS to measure disease progression and to develop new therapies aimed at mitigating motor neuron degeneration in ALS.

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C38 ZEBRAFISH TO IDENTIFY MODIFIERS OF ALS

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Keywords: zebrafish, EphA4

Using a previously established zebrafish model for ALS, we performed a morpholino-based screen to identify genes that modify the phenotype. Knockdown of the fish orthologue of EphA4 rescued the axonopathy induced by mutant SOD1. Similarly, decreasing EphA4 expression or EphA4 antagonists attenuated the motor neuron degeneration in mouse and rat models. This protective effect was particularly seen on large motor neurons, which are known to be vulnerable in ALS. These large cells express high levels of EphA4, and this receptor inhibits their sprouting capacity. In patients with ALS, lower expression of EphA4 was associated with later age at onset and longer survival. In addition, rare loss-of-function mutations in the EphA4 gene are associated with unusually long survival.

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C39 LOSS OF TDP-43 CAUSES HYPOPERFUSION AND MUSCLE DYSTROPHY

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Keywords: TDP-43, mutant, zebrafish

Autosomal dominant mutations in the Tar DNA binding protein of 43 kDa (TDP-43; TARDBP) cause Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration with ubiquitin and TDP-43 positive inclusions (FTLD-TDP). Gain- and loss-of-function of TDP-43 are currently discussed as possible disease mechanism.

We generated zebrafish TDP-43 loss of function zebrafish mutants by zinc finger nuclease genomic editing to determine the physiological function of TDP-43. The transparent, easily accessible zebrafish embryos facilitate the identification of phenotypes and signaling pathways associated with the loss of TDP-43 function. Homozygous loss-of-function mutations in zebrafish *tardbp* show no morphological phenotype due to compensation by a splice variant of *tardbpl* (Tar DNA binding protein of 43 kDa like), a second zebrafish orthologue of human *TARDBP*. *tardbp* and *tardbpl* double homozygous mutants show a dramatic muscular dystrophy like phenotype. Additionally they display strongly reduced blood circulation and a dramatic mispatterning of intersomitic vessels, impaired spinal motor axon outgrowth and early death. A quantitative proteomic approach identified misregulated proteins in TDP-43 loss-of-function zebrafish mutants. Strikingly, similar misregulation of these proteins is observed in the frontal cortex of FTLD-TDP patients, suggesting a loss-of-function disease mechanism.

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C40 CHEMICAL GENETIC SCREENS FOR *IN VIVO* TDP-43 MODIFIERS AND ALS DRUG DISCOVERY

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Keywords: TDP-43, chemical genetics, animal models

Background: Mutations in TDP-43 and FUS are linked to ALS, but our understanding of the molecular mechanisms leading to pathogenesis is unclear. In the absence of well-defined *in vitro* mechanisms for drug discovery, *in vivo* models offer the best chance of identifying neuroprotective molecules.

Objectives: Our laboratories have developed animal models for TDP-43 and FUS motor neuron toxicity using worms (*C. elegans*) and zebrafish (*D. rerio*). These models recapitulate essential features of the ALS and are invaluable for learning more about pathogenic mechanisms and are highly amenable for chemical screening.

Methods: Transgenic worms expressing human mutant TDP-43 show motility defects leading to progressive paralysis that is readily apparent when the animals are grown in liquid culture. Adult TDP-43 worms were grown in 96 well plates and incubated with compounds. Compounds that rescued motility defects were then tested in the zebrafish model. Compounds that improved motor phenotypes also reduced neurodegeneration in all models.

Results: We screened approximately 3,700 FDA-approved molecules and identified 17 compounds that consistently reduced TDP-43 toxicity in worms and fish. These compounds also reduced FUS toxicity in our worm and fish models. Within this group are compounds linked to the Endoplasmic Reticulum stress response as well as a number of neuroleptics. Our data suggests that protein misfolding and synaptic dysfunction contribute to TDP-43 toxicity.

Discussion: Our chemical genetic screens in worms and fish models has identified a number of compounds that reduce motor neuron dysfunction and cell death caused by mutant TDP-43 and FUS proteins. As many of these compounds are well characterised, they help provide information on pathogenic mechanisms for TDP-43 and FUS toxicity *in vivo*. Furthermore, these compounds provide early leads for ALS drug discovery and development. Lastly, since a number of these compounds are already FDA–approved, they have clinical applications.

Conclusions: This is the first chemical screen in multiple model organisms against TDP-43 and FUS toxicity. Our screen has high-throughput potential, and our initial findings

have been highly informative about cellular mechanisms of toxicity. An update of our work will be presented.

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C41 FUS IN ALS: NOVEL CELLULAR AND ANIMAL MODELS OF MOTOR NEURON DISEASE

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Keywords: FUS, Drosophila, locomotion

Mutations in the RNA-binding protein Fused in sarcoma (FUS) have been shown to cause familial amyotrophic lateral sclerosis (ALS). However, it is not clear how mutations of FUS lead to motor neuron degeneration in ALS. We have established a *Drosophila* model to examine the toxicity of FUS. The expression of wild-type FUS or FUSR521G induced progressive toxicity in a dose- and age-dependent manner in a variety of tissues, including eyes and neurons. In particular, the expression of FUS or FUSR521G in motor neurons significantly impaired the locomotive ability of fly larvae and adults. The presynaptic structures in NMJs were disrupted and the motor neurons in the ventral nerve cord (VNC) were disorganized and underwent apoptosis as evidenced by nuclear staining and TUNEL assay. Strikingly, FUS lacking its C-terminal nuclear localization sequence (NLS) has much less effects compared to those caused by FUS or FusR521G, suggesting that nuclear localization is required for FUS toxicity. Moreover, we discover that the loss of *caz* in *Drosophila* leads to severe growth defects in the eyes and ventral nerve cords. The loss of *caz* in motor neurons leads to locomotive disability and NMJ disruption but does not induce apoptotic cell death. The finding suggests that although both the overexpression and the deletion of FUS/Caz cause similar phenotypes, the underlying mechanisms for the gain-of-function and loss-of-function toxicity are likely to be different. Thus, some cases of the diseases caused by FUS mutations are likely due to the loss of function *in vivo*. We have recently identified phosphorylation of FUS to be a critical post-translational event that regulates FUS toxicity. We will present these findings in the meeting.

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C42 FUSOPATHY IN CELLS AND TRANSGENIC MICE EXPRESSING AN AGGREGATION-PRONE FORM OF HUMAN PROTEIN

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Keywords: FUS/TLS, aggregation, transgenic mice

Background: FUS and TDP-43 proteinopathies are characteristic features of certain forms of ALS and FTLD. These proteins share striking structural similarity, though the order of functional domains in the FUS protein is inverse to that in TDP-43. Approximately 25 kDa C-terminal fragments of TDP-43 are commonly present in association with histopathological inclusions in the nervous system of patients with ALS-TDP. There is a growing body of evidence that these truncated variants of TDP-43 contribute to the development of neurodegenerative changes.

Objectives: Here, we addressed the question of whether a truncated variant of FUS with a domain composition similar to that of truncated TDP-43 would trigger proteinopathy in cell culture models and in transgenic mice.

Methods: Various isoforms of human FUS protein bearing amino acid substitutions or C-terminal truncations were expressed in SH-SY5Y human neuroblastoma cells, and their intracellular distribution and co-localisation with various markers were assessed using immunofluorescence. Transgenic mice expressing a truncated variant of FUS under the control of a Thy1 promoter were produced and a detailed characterisation of their phenotype was carried out.

Results: In agreement with previously reported observations, in SH-SY5Y cells, the expression of FUS variants lacking functional nuclear localisation signal triggered the formation of stress granules and the accumulation of expressed proteins within them. However, the truncated variant of FUS formed different types of cytoplasmic structures in SH-SY5Y and other types of cultured cells. These structures displayed typical morphological and biochemical characteristics of intracellular inclusions consisting of aggregated proteins. Transgenic mice expressing C-terminally truncated FUS in the majority of neurons developed neuronal pathology at the age of 3–5 months, which led to severe disability and death within 1–2 weeks after the onset of clinical signs. Multiple FUS-positive pathological cytoplasmic inclusions were observed in lower and upper motor neurons. The terminal stage of the disease was characterised by severe damage and loss of myelinated motor fibres in the ventral roots with sensory fibres in the dorsal roots much less affected. The loss of lower motor neurons was selective to certain discrete populations and coincided with the degree of neuroinflammation in the corresponding region.

Discussion and conclusions: C-terminal truncation of FUS protein dramatically increases its ability to aggregate and form cytoplasmic inclusions. The expression of the truncated form of FUS protein triggers FUSopathy in cultured cells and transgenic mice. Our FUS transgenic mouse model recapitulates many key features of ALS.

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SESSION 7B CLINICAL TRIALS & TRIAL DESIGN

C43 RESULTS OF A PHASE 1, DOUBLE-BLIND, PLACEBO-CONTROLLED, DOSE-ESCALATION STUDY OF THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF ISIS 333611 ADMINISTERED INTRATHECALLY TO PATIENTS WITH FAMILIAL ALS DUE TO SOD1 GENE MUTATIONS

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Keywords: antisense, SOD1, phase I

Objective: To evaluate the safety, tolerability, and pharmacokinetics of four dose levels of ISIS 333611, an antisense oligonucleotide designed to inhibit SOD1 mRNA, delivered by intrathecal infusion.

Background: Mutations in SOD1 cause about 13% of familial ALS. In animal studies, delivery of ISIS 333611 to the CSF resulted in drug distribution to the brain and spinal cord, decreased SOD1 mRNA and protein levels in spinal cord tissue, and prolongation of survival in the $SOD1^{G93A}$ rat model.

Design and methods: A randomized, placebo-controlled Phase 1 safety trial of ISIS 333611 is currently in progress. ISIS 333611 is delivered by intrathecal infusion using an external pump over 12 h at increasing doses. Four cohorts of eight SOD1-positive ALS patients were studied (randomized six drugs; two placebos/cohorts). Safety/tolerability measures, ALSFRS-R, FVC, and neurological exams are assessed during the infusion and at 1, 8, and 29 days after the infusion. CSF and plasma drug levels are also measured.

Results: No dose-limiting toxicities were identified. There were no serious adverse events in ISIS33361-treated subjects. Adverse events were mild or moderate in severity and none were related to the dose level of ISIS333611. Most common AEs were related to delivery procedure. No drug-related neurological changes were observed. ALS-FRS and FVC measurements were only changed in those patients with rapidly progressing mutations. Re-enrollment was well tolerated CSF and plasma drug levels were consistent with levels predicted from preclinical studies.

Conclusions: ISIS 333611is well tolerated and the resulting CSF and plasma drug levels are as predicted. This is the first clinical study to report intrathecal delivery of an antisense oligonucleotide. Results from this study suggest that antisense oligonucleotide delivery to the CNS may be a viable therapeutic strategy for neurological disorders.

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C44 RESULTS OF PHASE 1 TRIAL OF SPINAL CORD TRANSPLANTATION OF NEURAL PROGENITOR CELLS IN ALS (THE NEURALSTEM, INC. TRIAL)

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Keywords: stem cells, transplant, spinal cord

We completed the FDA-approved Phase 1 trial of spinal cord transplantation of neural stem cells in 18 patients. Using a paradigm of "escalating risk", six patients received unilateral injections and six received bilateral injections into the lumbar cord (L2-L4). Following this, three new patients received unilateral injections into the cervical spinal cord (C3-C5). The final three patients are the same as those who previously received bilateral lumbar injections. At the time of this submission, one of these subjects has successfully undergone unilateral cervical transplantation and the remaining two subjects are scheduled for surgery. All procedures included 5 injections per side, 100,000 cells in 10 µl per injection. Thus, the range of doses was 500,000 cells in 5 injections to 1.5 million cells in 15 injections (patients 16–18). The proprietary injection apparatus was fixed to the patient's spine and so it was able to move simultaneously with any patient movement ("floating cannula"), avoiding lateral shear during the operation. All patients tolerated the surgical procedure with minimal perioperative or postoperative problems. One patient developed cervical kyphosis as a complication of multilevel laminectomy. There were no adverse events attributable to the cellular injections. Patients were immunosuppressed with a combination of tacrolimus and mycophenolate, which resulted in gastrointestinal distress in about a third of the patients. Clinical progression of disease continued in all patients except one, who showed remarkable improvement by both clinical and electrophysiological measures. At the time of this submission (June, 2012), there

were four deaths. The technique of real-time quantitative PCR (qPCR) was used to identify and quantify donor cell DNA in the spinal cord tissue of these deceased patients. The conference presentation will provide up-to-date information on progress as of December 2012. In summary, among patients with ALS, we have demonstrated the tolerability and safety of spinal cord transplantation with neural stem cells. Based on these findings, efficacy testing of this aggressive tactic as a therapeutic option for an otherwise untreatable neurodegenerative disease can now be pursued.

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C45 PHARMACOKINETICS AND INTERACTIVE EFFECTS OF THE FAST SKELETAL MUSCLE ACTIVATOR CK-2017357 AND RILUZOLE

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Keywords: clinical trial, muscle activator, pharmacokinetics

Background: CK-2017357 (CK-357) is a fast skeletal muscle activator that increases the efficiency of submaximal voluntary muscle contraction. In both single and multiple dose studies, CK-357 was well tolerated and showed encouraging trends on a variety of functional outcomes. After a single dose, CK-357 peak levels showed a linear relationship to dose level. As a CYP1A2 inhibitor, CK-357 increased plasma riluzole levels after a single dose.

Objectives: To determine the pharmacokinetics of repeated doses of CK-357 both in the presence and absence of riluzole, and to determine the effects of CK-357 at varying doses on plasma riluzole serum levels.

Methods: A total of 49 patients with ALS were treated; 24 patients were not taking riluzole; the remainder took a stable but reduced dose of riluzole 50 mg daily. Patients (n = off/on riluzole) received single daily doses of placebo (n = 6/7), 125, 250, or 375 mg of CK-357 (n = 6/6 for all 3 CK-357 groups) for 14 days. CK-357 and riluzole levels were measured on Days 1, 2, 8, and 15.

Results: Plasma levels of CK-357 achieved steady state by Day 8; 4 h after dosing on Day 8, the levels were approximately 70% higher than those observed at 4 h after the first dose on Day 1. CK-357 Cmax increased proportionally by dose with no apparent effect of riluzole on CK-357 Cmax. At 125 mg, 250 mg, and 375 mg daily, Cmax was 4.1 mcg/ml, 7.4 mcg/ml, and 12.7 mcg/ml, respectively, for subjects who were not on riluzole, and 6.0 mcg/ml, 8.4 mcg/ml, and 13.5 mcg/ml for those subjects who were on riluzole. In contrast, CK-357 approximately doubled riluzole levels similarly across all dose groups. Adverse event frequencies were not altered by the presence of riluzole at any dose of CK-357.

Conclusions: CK-357 had predictable linear kinetics at the repeated doses used in the current study, reaching steady state within 1 week. CK- 357 plasma levels were not affected by the presence of riluzole. Riluzole levels were increased by CK-357, with plasma levels increasing approximately two-fold across all dose levels of CK-357. However, no adverse events were reported during this study, and this was attributable to higher riluzole levels, with the daily riluzole dose reduced to 50 mg daily. These results suggest that CK-357 and riluzole may be given safely in combination.

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C46 A PHASE 2–3 TRIAL OF OLESOXIME IN SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: olesoxime, clinical trial, phase 3

Background: Olesoxime has demonstrated neuroprotective and neuroregenerative properties in extensive *in-vitro* and *in-vivo* (SOD1G93A mice) studies. It targets mitochondrial dysfunction that might have an important early role in the development of ALS. It has been tested in Phase 1a and 1b without safety concerns.

Objective: To assess the efficacy and safety of olesoxime in ALS patients treated with riluzole.

Methods*:* A double-blind, randomized, placebo-controlled, multicenter trial of 18 months duration was conducted in 512 subjects with probable or definite ALS receiving 330 mg olesoxime daily or matching placebo. The primary intention-to-treat analysis was 18-month survival. Secondary outcomes were on survival without tracheostomy, invasive ventilation or permanent non-invasive ventilation and on rates of deterioration of ALS Functional Rating Scale-Revised (ALS FRS-R, focusing on the 9-month assessment), Slow Vital Capacity (SVC) and muscle strength. Blood levels, safety and tolerability of olesoxime were also assessed.

Results: At 18 months, 154 of the 512 ITT patients had died (79 of 253 placebo, 75 of 259 olesoxime). The estimated overall survival according to Kaplan-Meier analysis was 67.5% (95% CI, 61.0 to 73.1%) in the placebo group and 69.4% (95% CI 63.0 to 74.9%) in the olesoxime group; hence survival was not significantly different between treatment arms (p = 0.71, stratified bulbar/spinal log-rank). Sensitivity analyses and other efficacy endpoints evaluated were also negative, with the exception of a small difference in ALS FRS-R global score at 9 months in favor of olesoxime (p = 0,0242; F test) but not after 18 months in either the bulbar or spinal sub-populations. Expected olesoxime plasma exposure was achieved in 97.3% of evaluable olesoxime-treated patients, with high inter-patient variability. Overall survival was not significantly different between the three pre-specified olesoxime exposure levels based on trough concentration. Treatment of this ALS population with combined riluzole and olesoxime did not raise any safety concerns.

Conclusions: Olesoxime, although well tolerated, did not show any beneficial effect in ALS patients treated with riluzole.

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C47 PHASE II SAFETY AND EFFICACY OF NP001: A NOVEL IMMUNE REGULATOR FOR ALS

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Keywords: biomarkers, clinical trial, treatment

Background: Abnormal inflammatory macrophages (AIM), systemically and locally in the CNS, are implicated in ALS progression. Although the exact trigger(s) are unknown, AIM activation is related to the rate of disease progression, suggesting that they reflect events relevant to ongoing CNS inflammation and neuronal death. CNS AIM perpetuate the ongoing pathogenesis through production of cytokines that attract and drive further AIM migration into the CNS. NP001, a novel immune regulator, lowered disease-associated markers of AIM activation *in vitro*, and is thus hypothesized to slow the progression of ALS. In a recent phase I trial in patients with ALS, NP001 reduced blood AIM inflammatory biomarkers in a dose-dependent manner.

Objectives: To assess the safety and efficacy of NP001 in a phase II trial.

Methods: A total of 136 patients, at 17 sites in the US, were enrolled in a randomized, double-blind, placebo-controlled study. Patients met key entry criteria: FVC > 70%, onset of weakness < 3 years, and no immune modulator therapy within 3 months. Patients were randomized 1: 1: 1 to receive NP001 1mg/kg/dose, 2 mg/kg/dose or placebo intravenously. Study drug was given over a 6-month treatment period as an induction cycle of 5 consecutive daily doses followed by 5 monthly cycles of 3 consecutive daily doses. Following 6 months of infusions, patients were seen on a monthly basis for 3 months to assess the durability of effect. The primary efficacy assessment was ALSFRS-R slope over the 6-month treatment period. Safety assessments were conducted throughout the trial and an Independent Data Monitoring Board reviewed the safety data in an ongoing fashion. The blood inflammatory biomarkers, wrCRP and MCP-1, were assessed at baseline and on a monthly basis during the treatment and follow-up period. The primary efficacy analytic approach was a general linear mixed effect model to assess slope. All patients who received the study drug were included in the safety analyses.

Results: A total of 136 patients, with a mean age of 54 years, were randomized. The mean baseline ALSFRS-R was 38. Most patients (93%) had sporadic ALS, 18% had bulbar onset, and 73% were on concomitant riluzole. A total of 115 patients completed treatment, and 112 are continuing to receive treatment in the follow-up period. A total of 16% of patients discontinued early, including five patients who died due to disease progression. Only 3% of patients discontinued due to adverse events. The incidence of infusion-related adverse events was low. Discussion: NP001 was generally safe and well tolerated. The effects of NP001 on disease progression and blood inflammatory biomarkers will be discussed.

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C48 SIX QUESTIONS FROM THE ALSFRS CONVEY THE SAME PROGNOSTIC SIGNIFICANCE FOR SURVIVAL AS THE TOTAL SCORE

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Keywords: ALSFRSr, abbreviated ALSFRS-6

Background: The ALS Functional Rating Scale (ALSFRSr) is widely used in clinical practice and in clinical drug trials as an outcome measure. It has been validated for phone-, internet-, and self-administration. Rapid progression conveys a negative prognosis for survival. Despite efforts to standardize the technique of administration, concern has arisen about adapting the ALSFRS with evolving therapeutic interventions, most recently, diaphragm pacing. Moreover, there is ambiguity about scoring in the setting of functional improvement as a result of therapy, for example, reduction in sialorrhea due to botulinum toxin injection. Finally, some functional responses are confounded by patient choice (e.g. not attempting to climb stairs when physically capable, refusing noninvasive ventilatory support despite a low percentage of FVC, etc). These potential imprecisions in scoring prompted a quest for a subset of questions that would be free of patient choice or therapeutic benefit, more clearly measure the functional performance in ALS patients, and still retain prognostic significance for progression and survival.

Objectives: To evaluate the performance of a subset of six questions from the ALSFRSr in measuring progression and predicting survival.

Methods: This is a secondary analysis of the BDNF 930121c dataset (n = 1135 patients rated monthly, 10 question ALSFRS) and the minocycline dataset (n = 409 patients with ≥ 4 visits, 12 question ALSFRSr). The 6 questions (ALSFRS-6) chosen were: Speech (Q1), Handwriting (Q4), Dressing and Hygiene (Q6), Turning in Bed (Q7), Walking (Q8), and Dyspnea (Q10, Q10a). In the BDNF analysis, the ALSFRS-6 score (range, 0–24) was compared to the ALSFRS score (range, 0–40) and the ALSFRS-Rem (remainder; range, 0–16) with univariate analysis (Log rank statistic) and multivariate analysis (Cox proportional hazards model). Using the minocycline dataset, the rate of decline in the ALSFRS-6 score over 4 months was compared to the ALSFRSr score (range, 0–48). Hazard ratios for death using each score were compared.

Results: The ALSFRS-6 score was highly correlated with the ALSFRS at baseline ($r = 0.959$, $p < 0.001$) or with the slope over 4 months ($r = 0.89$). The ALSFRS-6 declined 20.0% from baseline (mean ± SD; 8.4 ± 3.2) over 9 months (−2.2% per month). In the minocycline dataset, the prognostic significance for survival using Harrell's C statistic was 0.68 for the ALSFRS-6 vs 0.70 for the ALSFRSr. In the BDNF dataset, the Cox model indicated that the ALSFRS-6 was the main indicator of survival to 18 months.

Discussion: These data demonstrate that ALSFRS-6 conveys the same prognostic significance for survival as the entire 12-question instrument. The full ALSFRSr remains useful as "review of systems" in the clinical setting. The abbreviated ALSFRS-6 appears to be free of the limitation

choice on the part of the patient and symptomatic improvement that might conflict with accurate rating of the full scale. The ALSFRS-6 can be used and still retain linkage with previous studies utilizing the full-scale ALSFRSr.

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C49 A HEALTH STATE STAGING SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: health states, staging system, delphi process

Background: Initial symptoms of amyotrophic lateral sclerosis (ALS) may appear as weakness or atrophy in limb or bulbar muscles, with later disease progression typically involving thoracic and abdominal muscles. It is hoped that treatments in development will preserve function and prolong survival in ALS patients. Clinical trials track ALS patients with functional rating scales, for example, ALSFRS-R. However, key health states that represent the natural disease course may better inform judgments for clinical and economic decision-makers. Clearly defined, discrete and mutually exclusive health state staging systems that reflect the biological progression of ALS have not been widely accepted or applied in clinical practice. A group led by King's College London described a staging mechanism based upon key milestones in ALS (involvement of first, second and third functional region, need for gastrostomy and need for non-invasive ventilation) (1). However, this system is limited in its definition of health states, as the levels of impairment in each region are not assessed.

Objectives: To derive a consensus-based ALS health state staging system that captures the progressive weakness and loss of function associated with deterioration of upper and lower motor neurons, incorporates the impact of regional involvement on patient functioning and recognizes the potentially different impacts on patients of bulbar versus limb onset. The staging system must be configured to include parameters that can be applied to modelling of treatment effects in clinical trials.

Methods: Functional involvement and impairment by region were defined using thresholds of ALSFRS-R subscale scores (bulbar, upper/lower limb and respiratory regions). Clinically relevant thresholds were decided on the basis of empirical analysis of HRQoL baseline data from the dexpramipexole phase III study (EMPOWER). A modified Delphi panel process was undertaken with the ALS Clinical Staging Task Force to obtain consensus on the expanded King's staging mechanism to allocate ALS patients to functional states. ALSFRS-R patient profile characteristics underrepresented at baseline in EMPOWER were allocated by the Task Force to functional states through an iterative modified Delphi panel process. Individual Task Force member opinions remained anonymous during the iterations, with the synthesized aggregate response provided to indicate points of agreement or disagreement. Consensus was defined as 75% agreement across responses from the Task Force.

Results: The findings of the Delphi panel and the resulting staging system will be presented. Application of this staging system to EMPOWER participants (N = 943) will be demonstrated.

Conclusion: The proposed ALS health state staging mechanism reached through consensus of the ALS Clinical Staging Task Force will inform research and clinical study design, questions of resource allocation and future health technology assessment.

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SESSION 8A CELLULAR DIVERSITY AND SELECTIVE VULNERABILITY

C50 MOLECULAR LOGIC OF CORTICOSPINAL MOTOR NEURON DEVELOPMENT, DEGENERATION, AND REGENERATION: WHAT DO WE KNOW ABOUT THE UPPER MOTOR NEURON?

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Keywords: corticospinal, upper motor neurons

This talk will discuss two central ideas relevant to ALS, both dealing with development of the 'upper motor neurons' involved:

1) That distinct molecular characteristics of corticospinal motor neurons (CSMN) and their related subsets of subcerebral projection neurons (SCPN), and potentially shared molecular components or pathways with spinal motor neurons (SMN), underlie the selective and specific vulnerability of these circuit partners among all the other thousands of unaffected neuronal populations.

2) That even subtle abnormalities in the development of CSMN/SCPN (and SMN, for that matter) might predispose these populations to vulnerability and later neurodegeneration in ALS/MND.

Given the heterogeneity of CNS neuronal subtypes, and the complexity of their connections, complex molecular controls regulate specification, differentiation, connectivity, and survival. CSMN/SCPN and other neocortical projection neuron populations are driven through stepwise and very specific steps of developmental refinement by a set of interacting developmental controls, mostly transcriptional regulators. These control key developmental processes including progenitor parcellation, subtype-specific differentiation, area identity, and axonal outgrowth. Loss-of-function and gain-of-function analyses for identified genes and molecules reveal a nested 'molecular logic' of progenitor-stage and postmitotic stage controls. These molecular controls not only act in multiple steps orchestrated over time, but are also parcellated in space, distinct at the neuronal subtype level in the same spatial position, and are 'state-dependent' for each separate function and combination. During the period over which progenitors build neurons, CSMN for example, there are many distinct progenitor types, each of which builds certain broader classes of neurons; CSMN/SCPN are closely related to other corticofugals—corticothalamics, corticostriatals, subplate, and all of the subcerebrals, including sensory projection neurons that project from layer five to occipital cortex. These are built from the same progenitors, but subsequent steps delineate each subset of them. Thus, during the development, evolution, and organization of CNS circuitry, even very subtle errors might be introduced that anticipate later degeneration of specifically vulnerable populations.

Recent work identifies that nonmotor SCPN also degenerate selectively in ALS model mice, potentially in part explaining nonmotor, cognitive, and sensory changes in MND. Further, multiple newly identified developmental genes of these types have now been identified from this work as human disease-related genes.

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C51 AAV2-MEDIATED RETROGRADE TRANSDUCTION OF CORTICOSPINAL MOTOR NEURONS REVEALS INITIAL AND SELECTIVE APICAL DENDRITE DEGENERATION IN ALS

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Keywords: AAV, corticospinal motor neuron degeneration, neuroinflammation

Background: Development, assessment, and application of novel approaches using adeno-associated virus (AAV) could provide safe, long-term expression of therapeutic genes in the central nervous system (CNS). AAV vectors have very low immunoreactivity in humans and there are currently several CNS trials exploring their therapeutic potential for Parkinson's disease, Canavan's disease, and Alzheimer's disease. Their application in amyotrophic lateral sclerosis (ALS) treatment has not been overlooked. Early studies demonstrated that AAV-IGF could be retrogradely transported to spinal motor neurons after injection into muscle. However, corticospinal motor neurons (CSMN), the cortical component of the motor circuitry that degenerates in ALS, have not been studied in detail, and AAV-mediated retrograde transduction of CSMN is poorly understood.

Objectives: To selectively target CSMN within the cerebral cortex without affecting other neuron populations or circuitries as a means to establish new and effective therapeutic approaches in ALS.

Methods: In this study, we investigated whether CSMN would be transduced upon AAV injection from the corticospinal tract (CST) that lies within the dorsal funiculus of the spinal cord and tested seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbor the eGFP gene for their retrograde transduction efficiency.

Results: Our studies revealed that AAV2-1, AAV2-2, AAV2-5, AAV2-6, and AAV2-9 retrogradely transduce CSMN with different efficiencies, but AAV2-7 and AAV2-8 fail to transduce

CSMN upon CST injection. Among all AAV serotypes tested, AAV2-2 showed the highest transduction level. In addition, retrograde transduction of CSMN in hSOD1^{G93A} transgenic ALS mouse, which show progressive CSMN degeneration, revealed selective apical dendrite degeneration and spine loss especially in layer II/III of the motor cortex, where CSMN function is heavily modulated. Our results also demonstrate precise cellular interactions of CSMN with microglia and astrocyte at both apical dendrite and soma level.

Discussion: This study provides a valuable therapeutic strategy to deliver genes of interest specifically into CSMN in the cerebral cortex without affecting other neurons or other circuitries and identifies AAV2-2 to be a potential tool for future gene delivery approaches. Moreover, our studies reveal, for the first time, the early signs of cellular degeneration in CSMN to start from the apical dendrite with pronounced loss of spines and apical dendrite degeneration. The presence of activated astrocytes and microglia, especially in close contact with degenerating apical dendrites, suggests their important role during disease progression.

Conclusions: Our findings both allow future cell type-specific gene delivery approaches to CSMN by using AAV2-2-mediated retrograde transduction and identify cellular basis of CSMN vulnerability in ALS.

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C52 ABERRANT NEUREGULIN1 SIGNALING IN ALS PATIENTS WITH UPPER MOTOR NEURON SIGNS

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Keywords: corticospinal tract degeneration, myelin-axon loss, neuregulin

Background: ALS involves both upper and lower motor systems, but how the upper and lower motor systems are linked during disease progression is not known. We recently found aberrant neuregulin1 (NRG1) signaling with NRG1 receptor activation on activated microglia in the ventral horn of both ALS patients and SOD1 mice, suggesting a common pathological mechanism (1). However, it is not clear whether this signaling system also plays a role in patients with upper motor neuron (UMN) features, where patients with ALS show significant myelin-axon loss in the corticospinal tracts (CSTs) that is not readily seen in the SOD1 mouse.

Objectives: In order to focus on the UMN system feature, we compared the degree of CSTs degeneration and NRG1 receptor activation in the presence or absence of UMN symptoms in well-characterized postmortem cervical, thoracic, and lumbar spinal cords regions to determine the anatomical and functional relationships of the connection between upper and lower motor neuron (LMN) systems in ALS patients.

Methods: NRG1 expression and NRG1 receptor activation on activated microglia were measured in the different levels of CST from ALS patients with and without clinical UMN symptoms. The degree of myelin/axonal loss in CST regions was quantified.

Results: Only patients with clinical upper neuron symptoms showed CST degeneration; however, when present, all three spinal cord regions were affected (cervical, thoracic, and lumbar). Quantification of axon density showed a loss of axons of different diameters in the lateral and ventral CST, but not in other tracts including the dorsal columns. Even though all patients showed activated microglia with NRG1 receptor activation in the ventral horn, only those patients with CST degeneration showed increased microglial activation co-localized with NRG1 receptor activation. These same regions also showed increased NRG1 protein expression.

Discussion and conclusions: Our current findings show that only patients with UMN signs show focal degeneration of the CST and that when it occurs, the entire tract is affected. This differs from recently published findings showing gradients of LMN loss as a function of disease progression in the ventral horn that often has greater effects on the portion of the spinal cord where the disease starts (2). The persistent activation of NRG1 receptors on activated microglia in the degenerating CST as well as the ventral horn may result from altered axoglial signaling patterns in ALS that could promote disease progression and serve as a potential therapeutic target to treat both LMN and UMN disease.

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C53 IDENTIFICATION OF NEUROTROPHIC FACTORS FOR ALS-RELEVANT MOTOR NEURON SUBSETS BY A NOVEL FACS-BASED APPROACH

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Keywords: neurotrophic factors, motor neuron subsets

Neurotrophic factors (NTF) represent promising therapeutic candidates for human ALS since they can enhance motor neuron survival during normal development and in rodent ALS models. Studies in knockout mice, however, suggested that subsets of motor neurons differ in their survival response to NTF. To identify NTF for ALS-relevant motor neuron subsets, we here used a combination of novel flow cytometry, fluorescent-activated cell sorting (FACS) and transcriptomic techniques.

Motor neurons innervating limb, axial and abdominal muscles were identified in mouse spinal cord through the combinatorial expression of transcription factors ISL1/2, HB9, FOXP1, LHX1/2, LHX3 and OCT6 by using double-color flow cytometry. Limb motor neurons – which are vulnerable in ALS – as well as axial motor neurons – which are relatively resistant – were then isolated by FACS. Both motor neuron subsets were obtained with high yield and exquisite purity, seeded into 96 well plates, cultured in the presence of the neurotrophic factors BDNF, NT-3, LIF, CNTF, CT-1, GDNF, Neurturin, Artemin or IGF and monitored for survival. Dose-finding experiments revealed distinct survival responses of limb and axial motor neurons to HGF and

CNTF. In line with these data, microarray-based gene expression profiling, immunoblot and *in situ* hybridization analyses identified differential expression of the HGF receptor c-Met and the CNTF receptor Lifrβ in subsets of limb and axial motor neurons, respectively.

In conclusion, this approach identifies those neurotrophic factors that are critical for the survival of ALS-relevant motor neurons and thereby provides a rationale for testing selected NTF or NTF combinations in preclinical ALS trials.

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C54 UNRAVELLING THE ENIGMA OF SELECTIVE VULNERABILITY IN NEURODEGENERATION: MOTOR NEURONS RESISTANT TO DEGENERATION IN ALS SHOW DISTINCT GENE EXPRESSION CHARACTERISTICS

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Keywords: Selective vulnerability, oculomotor neuron, excitotoxicity

Background: A consistent clinical feature of amyotrophic lateral sclerosis (ALS) is the sparing of eye movements and the function of external sphincters, with corresponding preservation of motor neurons in the brainstem oculomotor nuclei, and of Onuf's nucleus in the sacral spinal cord. Studying the differences in properties of neurons that are vulnerable and resistant to the disease process in ALS may provide insights into the mechanisms of neuronal degeneration and identify targets for therapeutic manipulation.

Objectives: 1) To use microarray analysis to determine the differences in gene expression between oculomotor and spinal motor neurons isolated from the midbrain and spinal cord of neurologically normal human controls. 2) To verify the most significant changes found in two other species, and by functional studies.

Methods: Spinal motor neurones were isolated by laser capture microdissection from the oculomotor nucleus and lumbar spinal cord of frozen CNS tissue, donated to the Sheffield Brain Tissue Bank by neurologically normal control subjects. RNA was extracted, amplified, and hybridized to Affymetrix expression arrays. Differential gene expression between oculomotor and spinal motor neurones was determined using the bioconductor package, Puma. These results were compared to the transcriptional profiles of oculomotor nuclei and spinal cord from rat and mouse, obtained from the GEO omnibus database. Patch clamp recording in acute spinal and brainstem slices was performed to confirm the functional significance of differential expression of GABA and glutamate receptors, between resistant and vulnerable motor neurone subtypes.

Results: A total of 1757 named genes were significantly ($p < 0.001$) differentially expressed between oculomotor and spinal motor neurones, and these were enriched for the functional categories of synaptic transmission, ubiquitin-dependent proteolysis, mitochondrial function, transcriptional regulation, immune system functions, and the extracellular matrix. Marked differences were seen, across the three species, in genes with a function in synaptic transmission, including several glutamate and GABA receptor subunits. Using patch clamp recording, we showed that oculomotor neurons exhibit a reduced AMPA-mediated inward calcium current and a higher GABA-mediated chloride current than spinal motor neurons do.

Discussion: Resistant oculomotor neurones have a distinct transcriptional profile. These differences in gene expression may reflect their diverse embryological origin, the different milieu in the brainstem, or differences in the structure and function of motor units of ocular muscles, compared to other skeletal muscles. Amongst the differences in gene expression observed are characteristics that render oculomotor neurons resistant to the degenerative process in ALS. The most significant changes identified in gene expression profiling were in GABA and glutamate receptor subunits, and we confirmed in functional studies that oculomotor neurones show changes in AMPA- and GABA-mediated currents, which would predict a lower susceptibility to excitotoxicity.

Conclusions: The findings suggest that reduced susceptibility to excitotoxicity is an important determinant of the relative resistance of oculomotor neurons to degeneration in ALS.

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SESSION 8B CLINICAL REGISTERS AND EPIDEMIOLOGY

C55 ALS/MND PATIENT REGISTRIES/ REGISTERS – WHAT ARE YOU TRYING TO DO WITH THE INFORMATION?

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Keywords: population-based, registers, registries

ALS/MND is the degenerative disease of the motor neuron network. The clinical phenomenology can be explained, in most patients, by site of onset, spread by contiguity independently at spinal and cortical levels, relative involvement of different motor neuron types (lower, pyramidal, or prefrontal), and the rate of disease progression. The precise method of spread of disease within the motor neuron network has yet to be elucidated: a role for mis-folded proteins has been proposed. Triggering disease onset has been attributed to interactions between genetic, environmental, and age-dependent factors. Predisposing genes have been identified in many familial and some sporadic cases. Environmental risk factors have been more elusive.

Combining information from patients may permit generation of descriptive and analytic epidemiological information. Descriptive epidemiology may be used to compare populations and track changes over time. Analytical epidemiology requires comparing patients to an appropriate reference group, and may be used to identify risk factors for disease occurrence. Population-based registers (registries), rather than referral-based or self-selected case series, are needed in order to deliver on these expectations.

This presentation will compare ALS/MND patient registers that have been established in Europe and North America. It will describe their declared goals and the results that they have reported. It will discuss reasons for the successes of some registers and the challenges faced by others. It will conclude by suggesting realistic expectations from population-based registers and by proposing areas where standardizing register methodology may improve the ability to compare results among registers.

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C56 IDENTIFYING ASSOCIATIONS BETWEEN PRESCRIBED DRUGS AND SURVIVAL OF ALS PATIENTS USING MEDICARE DATA

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Keywords: epidemiology, database, survival

Background: Medicare data have been used to study many healthcare-related questions. Individuals diagnosed with ALS are immediately eligible for Medicare. This provides a large database of patients with ALS which includes information on prescription drugs.

Objectives: To demonstrate the feasibility of using Medicare claims data to determine if there are particular classes of drugs associated with survival in ALS.

Methods: In 2007, there were 14,116 patients with a part B claim diagnosis of ALS. We obtained all 519,299 part B claims and 303,326 Part D drug claims from 2007, as well as basic demographics including age, gender, date of enrolment and, if deceased, their date of death. For beneficiaries <65 years of age when enrolled in Medicare, we used the date of enrolment as a proxy for disease onset. Of the 14,116 beneficiaries, 5912 (42%) were enrolled prior to turning 65 and of these 3,083 had Part D drug claims. In order to improve the accuracy of the ALS diagnosis, we restricted the analyses to a cohort that was prescribed riluzole and did not also have a diagnosis of Parkinson's disease or MS. This analysis cohort consisted of 744 beneficiaries with a mean survival of 78 months. We computed survival time from the date of enrolment until the date of death or until January 2011. We used CHAID and COX proportional survival models with step-wise regression to find potential associations between drug classes and survival.

Results: The age ranged from 24 to 81 (mean 53), 56% were male. As of January 31st, 2011, 248 of these patients were still alive. We found several drug classes that were associated with longer survival, including loop diuretics, cyclooxygenase 2 (COX2) inhibitors, cephalosporins and selected statins. When the drug classes of interest were assessed using the multivariable COX model adjusting for patient demographics and relevant comorbidities, COX2 inhibitors, loop diuretics and nitrates and nitrites continued to show statistically significant impact on survival. The presence of some comorbidities, in particular, hypertension, diabetes and hypercholesterolemia, was found to be associated with longer survival.

Discussion: While the use of large administrative databases has limitations, we were able to identify several classes of

drugs associated with improved survival in ALS. Loop diuretics and nitrates are two drug classes associated with improved survival in ALS, which have not been previously described. The comorbidities of diabetes, hypertension and hypercholesterolemia may be serving as an indicator of high body mass index, which has been shown to positively impact survival.

Conclusions: We found several novel classes of drugs that are associated with improved survival in ALS. Further investigation using other ALS cohorts is warranted to confirm our findings.

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C57 INCIDENCE OF VENOUS THROMBOEMBOLIC EVENTS AMONG ALS PATIENTS IN A US HEALTH INSURANCE CLAIMS DATABASE

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Keywords: comorbidities, venous thromboembolism, pulmonary embolism

Background: Immobility may put amyotrophic lateral sclerosis (ALS) patients at increased risk for venous thromboembolic (VTE) events. Data from clinical trials and tertiary clinics, which may not be representative of all ALS patients, suggest that the incidence of VTE is higher than the general population (1–4).

Objective: Estimate the risk of VTE events in ALS patients compared to controls within the i3 InVision Data Mart Multiplan database.

Methods: Two cohorts of patients ≥ 18 years of age were included in this analysis: ALS patients (n = 4102, any patient with 1 inpatient or 2 outpatient medical claims containing ICD-9 code 335.20) and controls (n = 65,000 randomly selected patients with no medical claims for ALS (ICD-9 code 335.20) or other motor neuron diseases (CD-9 codes 335.2, 335.21, 335.22, 335.23, 335.24, 335.29)). VTE events were defined as any inpatient or emergency room medical claim with the following ICD-9 codes: 415.1x (pulmonary embolism and infarction), 451.xx (phlebitis and thrombophlebitis), and 453.xx (other venous embolism and thrombosis). Pulmonary embolism (PE) and deep vein thrombosis (DVT) were analyzed separately as secondary outcomes. Poisson regression was used to calculate incidence rates, while Cox proportional hazards models were used to calculate hazard ratios (HR).

Results: The crude incidence rate of VTE in ALS patients and controls was 1549.2/100,000 person-years (PYs) and 130.4/100,000 PYs, respectively. The HR of VTE in ALS patients was 12.3 (95% CI: 9.2, 16.4). The crude incidence rate of PE in ALS and controls was 192.9/100,000 PYs and 33.0/100,000 PYs, respectively, with a HR of PE in ALS of 6.4 (95% CI: 3.2, 12.8). The crude incidence rate of DVT in ALS and controls was 1473.5/100,000 PYs and 102.0/100,000 PYs, respectively, with a HR of DVT in ALS of 14.8 (95% CI: 10.8, 20.3). Results from the multivariate models will be presented at the conference.

Discussion and conclusions: ALS patients are at increased risk for VTE. The present analysis found an increased risk of VTE, PE, and DVT in ALS patients relative to the general population. Clinicians should be vigilant for signs of VTE in ALS patients.

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C58 MEDITERRANEAN DIET MODIFIES RISK OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: case-control study, risk factor, dietary pattern

Background: Dietary habits may influence pathophysiological mechanisms in sporadic amyotrophic lateral sclerosis (ALS), and, since they are modifiable, they may harbor potential preventive interventions.

Objective: We determined the relation between dietary patterns, identified by exploratory factor analysis, and the risk of sporadic ALS, adjusted for confounding factors and corrected for multiple comparison.

Methods: In a population-based case-control study in the Netherlands between 2006 and 2011, we studied the relation between dietary patterns, identified by exploratory factor analysis, and the risk of sporadic ALS, adjusted for confounding factors and corrected for multiple comparison. A food frequency questionnaire was used for data collection.

Results: A total of 747 patients and 2,385 controls were included. Two dietary patterns were independently associated with sporadic ALS. A dietary pattern characterized by a high intake of total, saturated, and monounsaturated fat, transfatty acids, and cholesterol and a low intake of alcohol was associated with an increased risk of ALS ($p < 0.001$). A pattern with a high intake of fibre, vitamin C, lycopene, flavonoids, and vegetable protein was associated with a decreased risk of ALS ($p = 0.001$).

Discussion and conclusions: The two identified dietary patterns point to a so-called "Mediterranean diet" and thus provide clues to pathophysiological pathways that might be involved in sporadic ALS. The positive association with fat intake may support a role for insulin resistance, while the

negative association with dietary antioxidants supports a role for oxidative stress in the development of sporadic ALS.

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C59 SEVERE VITAMIN D DEFICIENCY CORRELATES WITH WORSE ALS PROGNOSIS

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Keywords: vitamin D, prognosis, neuroprotection

Background: Vitamin D (VD) has been shown to modulate neurite outgrowth and to promote neuroprotection in different animal models. In clinical neurology, VD deficiency has also been related to vascular abnormalities and particularly an increased intima-media thickness. In ALS, all these elements are likely to increase disease severity. On the immunologic point of view, VD is able to participate to the Th1/Th2 imbalance through an action on T regulators (Treg). In ALS such a dysregulation of Tregs has been described and therapeutical intervention on Tregs has been shown to be able to modulate survival in transgenic SOD1 mice.

Objectives: To compare ALSFRS (Amyotrophic Lateral Sclerosis Functional Rating Scale) decline between three groups of ALS patients according to VD levels: normal (NVD), deficient (DVD) and severely deficient (SVDD).

Material and methods: VD levels were determined in 78 incident ALS patients. The following ALS criteria were collected: age of onset, site of onset, ALS duration, ALSFRS at the time of VD dosage and gender. VD collection was done between 2008 and 2011, and the results were retrospectively analyzed with regard to ALS evolution (ALSFRS is collected at each quarterly visit until end of follow up or death) and ALS severity (rate of ALSFRS decline, ROAD). Three groups were constituted according to VD levels: >75mmol NVD, >25mmol VDD, <25mmol SVDD. Data were analyzed using ANOVA followed by a student *t* test.

Results: Patients with SVDD had a four fold more important ROAD than NVD patients (1.278 pts/month vs 0.38, $p = 0.001$). The ROAD for VDD patients was intermediate (0.92 pts/month, ns vs other groups). At the time of database lock (April 2012), 80% of the SVDD group was already dead compared to 32 % for VDD patients and 23% for NVD ones, while they had, at the time of VD dosage, a shorter ALS duration (26, 30 and 36 months, respectively).

Conclusion: This study showed that patients with a SVDD have a four fold more rapid evolution than those with NVD levels. The exact reason for this may lie both in the role of VD on neurite outgrowth and in the role of VD on innate immune system. We believe that the potential of VD supplementation in ALS patients with SVDD has to be considered.

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SESSION 9A PHENOTYPIC CHANGE/ MODIFICATION

C60 DIFFERENT HUMAN COPPER-ZINC SUPEROXIDE DISMUTASE MUTANTS, SOD1G93A AND SOD1H46R, EXERT DISTINCT HARMFUL EFFECTS ON GROSS PHENOTYPE IN MICE

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Keywords: SOD1, gross phenotype, harmful effect

Amyotrophic lateral sclerosis (ALS) is a heterogeneous group of fatal neurodegenerative diseases characterized by a selective loss of motor neurons in the brain and spinal cord. Creation of transgenic mice expressing mutant Cu/Zn superoxide dismutase (SOD1), as ALS models, has made an enormous impact on the progress of ALS studies. Recently, it has been recognized that genetic background and gender affect many physiological and pathological phenotypes. However, no systematic studies focusing on such effects using ALS models other than SOD1(G93A) mice have been conducted.

To clarify the effects of genetic background and gender on gross phenotypes among different ALS models, we here conducted a comparative analysis of growth curves and lifespans using congenic lines of SOD1(G93A) and SOD1(H46R) mice on two different genetic backgrounds; C57BL/6N (B6) and FVB/N (FVB). Copy number of the transgene and their expression between SOD1(G93A) and SOD1(H46R) lines were comparable. B6 congenic mutant SOD1 transgenic lines irrespective of their mutation and gender differences lived longer than corresponding FVB lines. Notably, the G93A mutation caused more severe disease phenotypes than did the H46R mutation, where SOD1(G93A) mice, particularly on a FVB background, showed a more extensive body weight loss and an earlier death. Gender effect on survival also solely emerged in FVB congenic SOD1(G93A) mice. Conversely, consistent with our previous study using B6 lines, the lack of Als2, a murine homolog for the recessive juvenile ALS causative gene, in FVB congenic SOD1(H46R), but not SOD1(G93A), resulted in an earlier death of the mice, implying a genetic background-independent but mutation-dependent phenotypic modification. These results indicate that SOD1(G93A)- and SOD1(H46R)-mediated toxicity and their associated pathogenic pathways are not identical. Further, distinctive injurious effects resulted from different SOD1 mutations, which are associated with genetic background and/or gender, suggesting the presence of several genetic modifiers of disease expression in the mouse genome.

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C61 COMPARISON OF THE GENE EXPRESSION PROFILE IN LASER CAPTURED MOTOR NEURONS OF TWO SOD1G93A MOUSE STRAINS WITH DIFFERING DISEASE PHENOTYPES

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Keywords: gene expression profile, laser captured motoneurons, neuroimmunity

Background: Amyotrophic Lateral Sclerosis (ALS) is a clinically heterogeneous disease with a high variability in the rate of symptom progression even in familial cases associated with autosomal dominant SOD1 gene mutations. Recently, we have observed that SOD1G93A mice on different genetic backgrounds, C57BL/6J and 129S2/Sv, exhibit a clinical phenotype which differed consistently in terms of speed of symptom progression and life span, even though they carry the same number of human SOD1 transgene copies.

Objectives: We aimed to compare the gene expression profiles of the motor neurons of these two SOD1G93A mouse strains in order to discover the molecular mechanisms that may contribute to a the distinct phenotypes and to uncover factors undelying fast and slow disease progression.

Methods: Lumbar spinal motor neurons (MNs) from the two SOD1G93A mouse strains were isolated by laser capture microdissection and analyzed by microarray analysis at four different disease stages (presymptomatic; onset; symptomatic and endstage). Validation of significant changes was made using immunoblot of the ventral horns of spinal cord and the immunohistochemistry of spinal cord slices.

Results: We identified a marked difference in the motor neuron transcriptome between the two mice strains at the onset of the disease, with a dramatic gene downregulation in the rapidly progressive (129S2/Sv) compared to the slowly progressing mutant SOD1 mice (C57BL/6J) (1278 vs 346; $q < 0.01$). G93A-129S2/Sv mice exhibit a higher tendency to accumulate protein aggregates due to an impairment of specific pathways involved in misfolded protein degradation, as well as deficiencies in mitochondrial function and axonal transport. In contrast , gene ontology (GO) pathway analysis of the MN transcriptional profile from G93A-C57BL/6J mice, revealed a strong gene enrichment in relation to immune system processes compare to G93A-129S2/Sv mice. MNs from the more benign mutant strain exhibit strong complement activation, overexpressing genes normally involved in the physiology of immune cells such as MHCI, CD22 and CCL2, which are up-regulated respectively.

Discussion and conclusions: We demonstrated that the motor neurons of the slowly progressing mice, unlike those from mice with more severe phenotype, are able to activate a series of genes with neuroprotective properties including inflammatory genes. In contrast, the mice with a faster disease progression exhibit an increased tendency to accumulate protein aggregates due to a greater impairment of some pathways involved in misfolded protein degradation. These results may enable the identification of prognostic markers of the disease and the design of more specific therapeutic strategies for ameliorating the speed of progression of motor neuron injury.

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C62 THE ROLE OF THE TRANSCRIPTIONAL REGULATOR PGC-1A IN MODULATING THE ALS PHENOTYPE

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Keywords: PGC-1a, metabolism, genetic modifier

Background: Wasting and metabolic failure are important features of experimental and human ALS. The transcriptional co-activator PGC-1alpha is an important regulator of mitochondrial activity and biogenesis in many metabolically active tissues, including brain, muscle and fat. Recent clinical and experimental evidence from research into Huntington's disease and Parkinson's disease suggests that impaired function or activity of PGC-1alpha contributes to the pathogenesis of neurodegenerative disease spectrum disorders. In ALS transgenic mice general overexpression of PGC-1a can extend lifespan while over expression limited to muscle has no survival effect. Our data show that ALS transgenic mice in addition to their well-documented motor phenotype and weight loss show a variety of additional abnormalities suggesting involvement of PGC-1alpha-mediated metabolic control. In addition recent work from our collaborators suggests that different promotors drive PGC-1a expression in the brain and in the periphery.

Objectives: We aimed to investigate whether the lack of PGC-1alpha expression worsens the metabolic and motor phenotype of ALS transgenic mice, whether this has an effect on their survival and whether PGC-1a modulates the human disease course.

Methods: We crossbred PGC-1a-/- and SOD1 (G93A, high copy) transgenic mice to generate PGC-1a-/-;SOD1(G93A) mice. As controls we used PGC-1a-/-;SOD1(wt), PGC-1a+/+;SOD1(G93A) and wild-type mice. Body weight, body temperature and blood glucose levels were measured in regular intervals starting at the age of six weeks. Additionally we performed an evaluation of motor phenotype and string agility. To gauge the relevance of the PGC-1a system for the human condition we analyzed the effect of the novel PGC-1a SNPs on disease onset of a large cohort of >700 European ALS patients.

Results: Our data show that deletion of PGC-1alpha leads to an aggravation of the motor phenotype in ALS-mice as well as to a reduced life span in male SOD^{G93A}-mice. Dysregulation of body temperature is a common trait of SOD^{G93A}- and SOD^{G93A}-mice lacking PGC-1alpha expression, whereas fasting glucose levels of PGC1 alpha; SOD^{G93A} mice are reduced. Also, specific PGC-1a SNPs are correlated with an accelerated age of onset in European ALS patients.

Discussion and conclusions: Our results support a potential role of PGC-1alpha-mediated metabolic regulation in the pathogenesis of ALS in SOD^{G93A} transgenic mice. Importantly, our modifier study supports the relevance of the PGC-1a system for the disease course in human ALS patients.

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C63 ENHANCED PGC-1A ACTIVITY AND INCREASED MITOCHONDRIAL BIOGENESIS IN SKELETAL MUSCLE MAINTAIN MUSCLE FUNCTION THROUGHOUT DISEASE IN A MODEL OF INHERITED ALS

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Keywords: PGC1a, muscle, therapy

The mechanism underlying the premature degeneration and death of neurons during amyotrophic lateral sclerosis (ALS) is still unknown. Nevertheless, evidence from many experimental directions has supported the proposal that an important feature of ALS is damage to mitochondria. Mitochondrial dysfunctions, such as elevated levels of mitochondrially derived reactive oxygen species and deficits in mitochondrial respiration and ATP production have been reported, in motor neurons and skeletal muscles of ALS patients and transgenic mice constitutively expressing SOD1 harboring ALS-linked mutations. In these transgenic mice, retraction of motor axons at neuromuscular junctions is one of the earliest presymptomatic events, implying that muscle could be a putative primary source for mutant SOD1 mediated toxicity. This issue is still debated, although lowering SOD1 mutant synthesis in muscle (by viral delivered siRNA or by selective deletion of a mutant SOD1 transgene solely in muscle) does not affect disease course. Nevertheless, it should be emphasized that it is not known if enhancing the metabolic capacity and mitochondrial biogenesis of the muscle can protect against neuronal death in ALS mice. Indeed, improving motor performance through a regular exercise activity and/or by means of viral delivery of insulin growth factor (IGF) in the muscle or by a muscle restricted-expression of a localized IGF isoform delays the onset and extends survival of ALS mice. With this in mind, we have tested whether increasing

mitochondrial activity in skeletal muscles is an attractive target for therapeutic development in ALS. We now report that selective elevation of PGC-1α (a transcriptional co-activator that induces multiple effects on muscle, including increased mass and activity of mitochondria) in muscles of mice that develop fatal paralysis from an ALS-causing SOD1 mutant elevates PGC1α-dependent pathways throughout disease course. Mitochondrial biogenesis and activity are maintained through end stage disease, accompanied by retention of muscle function, delayed muscle atrophy and significantly improved muscle endurance even at late stages of disease. However, survival was not extended. Therefore, muscle is not a primary target of mutant SOD1-mediated toxicity, but drugs increasing PGC-1α activity in muscle represent an attractive therapy for maintaining muscle function during progression of ALS.

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C64 ACID SENSING ION CHANNELS (ASICS) CONTRIBUTE TO MOTONEURON DEGENERATION IN AN ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: acid sensing ion channels, SOD1 transgenic mice, therapeutics

Background: Mitochondrial dysfunction, Ca^{2+} overloading, and a local hypoxic/ischemic environment have been implicated in the pathophysiology of ALS and are conditions that may initiate metabolic acidosis in the affected tissue. We tested the hypothesis that acidosis, and, in particular acid-sensing ion channels (ASICs), are involved in the pathophysiology of ALS.

Objectives: Our aim was to investigate the role of acidosis and ASIC channels in the pathophysiology of ALS, and explore whether pharmacological inhibition of ASIC channels represents a new approach for the treatment of ALS.

Methods: We measured the intracellular pH of lumbar spinal cord samples in *SOD1* mice across disease progression by Neutral Red assessment. We determined whether *acidosis* contributed to neuronal death and whether ASIC1a was involved using *asic1a* deletion and ASIC1a blockade with PcTx1 *in vitro*. We generated *a mouse model of ALS* deficient for *asic1a and assessed* whether *asic1a* was involved in motoneuron degeneration in *SOD1* mice. We assessed whether acidosis is accompanied by changes in ASIC channel expression *in vivo* and in ALS patients. We next tested whether cross-inhibition of both ASIC1 and ASIC2 channels exerted an increased capacity to protect motoneurons against acidotoxic injury *in vitro*. We finally examined the effect of oral administration of ASIC channel inhibitors on lifespan, motor performance and motoneuron survival in the *SOD1* mouse.

Results: We found that acidosis increased across disease progression in the spinal cord of *SOD1* ALS mice. Moreover, motoneurons were selectively vulnerable to acidotoxicity *in vitro*. Acidotoxicity was partially reduced in *asic1a*-deficient motoneuron cultures, and crossbreeding of *SOD1* with *asic1a*-deficient mice delayed the onset and progression of motor dysfunction in *SOD1* mice. Interestingly, we also noted a strong increase in ASIC2 expression in motoneurons of *SOD1* mice and sporadic ALS patients during disease progression. Pharmacological pan-inhibition of ASIC channels with lipophilic amiloride derivative, 5-(N,N-Dimethyl) amiloride hydrochloride, potently protected cultured motoneurons against acidotoxicity, and, given post-symptom onset, a significantly improved lifespan, motor performance and motoneuron survival in *SOD1* mice (n = 24/group; age, gender (12 males/12 females), weight and litter-matched) in accordance with the most recent ALS guidelines for generating preclinical data (1).

Conclusions: Our data provide strong evidence for the involvement of ASIC channels in motoneuron degeneration, and highlight the potential of ASIC inhibitors as a new treatment approach for ALS.

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C65 ELECTRICAL IMPEDANCE MYOGRAPHY AND MUNE SHOW NO EVIDENCE OF RILUZOLE HAVING A THERAPEUTIC EFFECT IN SOD1 G93A MICE

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Keywords: motor unit number estimation, electrical impedance myography, riluzole

Background: Electrical impedance myography (EIM) and motor unit number estimation (MUNE) are two neurophysiologic biomarkers for evaluating ALS progression. Whether either is capable of identifying an actual treatment effect, however, is unknown. Since previous studies have given conflicting results as to the potential efficacy of riluzole in extending survival in SOD1 G93A mice, it remains possible that another indicator of disease progression, such as EIM or MUNE, could detect a treatment effect in these mice.

Objectives: 1. We sought to determine whether EIM or MUNE could detect a treatment effect in SOD1 G93A mice treated with riluzole versus controls. 2. We sought to compare how EIM and MUNE fared as biomarkers of disease onset and progression in this animal model.

Methods: Forty-seven SOD1 G93A animals were divided into two groups, one receiving riluzole in the drinking water, aiming for a dose of 0.2 mg/kg/day starting at six weeks of age. The groups were evenly divided between males and females. Animals underwent EIM, MUNE, and functional assessments weekly. Animals were sacrificed when they were no longer able to feed themselves due to complete hind limb paralysis.

Results: Treatment with riluzole was not found to prolong either the time of clinical disease onset, as judged by onset of leg tremors, or of survival (treated mean onset 118.3 days, untreated mean onset 117.7 days; treated mean survival 132.4 days, untreated mean survival 133.0 days). Rates of decline

for MUNE (11.4 units/week treated, 6.4 units/week untreated) and EIM phase-slope (1.15×10^{-3} degrees/kHz/week, treated, 8.37×10^{-4} degrees/kHz/week untreated) were also not different between groups ($p = 0.25$ and $p = 0.54$, respectively). Since no differences were observed, untreated and treated data were combined for the remaining biomarker analyses. First, EIM phase-slope correlated strongly with MUNE ($r = 0.78$, $p = 0.0141$). Significant changes in EIM phase-slope were identifiable at just nine weeks ($p = 0.0024$), whereas MUNE first showed a significant reduction at 14 weeks ($p = 0.028$). The rate of deterioration in EIM phase-slope ($r = 0.52$, $p < 0.001$) provided a stronger correlation with survival than MUNE ($r = 0.36$, $p = 0.012$). Finally, EIM phase-slope had a low coefficient of variation (CoV) in the rate of decline (0.34), far surpassing weight (CoV = 1.1) and MUNE (CoV 3.4), suggesting it would be highly sensitive to a treatment effect.

Discussion and conclusion: These EIM and MUNE data failed to show a therapeutic effect of riluzole in ALS SOD1 mice. However, these results do show the potential value of EIM in assessing ALS progression, through its early detection of disease onset, its rate of decline correlating to survival, and its low coefficient of variation in the rate of decline across animals.

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SESSION 9B MULTIDISCIPLINARY MANAGEMENT

C66 THE JOINT COMMISSION (TJC) DISEASE-SPECIFIC CARE CERTIFICATION (DSC) PROGRAM FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS) – A STRUCTURED MECHANISM DEVELOPED FOR PERFORMANCE MEASURE IMPLEMENTATION, AUDIT AND COMPLIANCE ASSESSMENT ALLOWING EVOLUTION FROM PROCESS-BASED TO PATIENT-OUTCOME-BASED IMPROVEMENT IN AN ALS MULTIDISCIPLINARY CENTER AVAILABLE INTERNATIONALLY

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Keywords: quality improvement, benchmarking, clinical audit

Background: Disease specific care certification (DSC) is a clinic-based process that assesses implementation of performance measures based on known ALS guidelines (1). The reiterative process was introduced to improve the quality of patient care by reducing variation in clinical processes reducing the risk of error, provide a framework for program structure and management using effective data-driven performance improvement, provide an objective assessment of clinical excellence, strengthen community confidence in the quality and safety of care, treatment and services, and potentially meet necessary regulatory requirements. The process is amenable to existing guidelines and performance measures recently developed by the American Academy of Neurology (2). Assessment of falls by patient history at each ALS clinic visit supplanted performance of Hendrich-II-Fall-Risk based on sensitivity analysis in 2009–2010. Assessment of falls was retired as an auditable performance measure as it became a standard for each clinical encounter and was replaced as an auditable performance measure by Respiratory Management Assessment (RMA)in 2011–2012.

Objective: Document compliance with core performance measures of the ALS DSC profile, demonstrate patient-specific outcome measure compliance and demonstrate addition of further performance measures in a large ALS clinic over 24 months (3,4).

Methods: Standardized performance measures: 1) Mini-Mental Status Examination(MMSE), 2)Patient Health Questionnaire (PHQ-2), 3)Patient Health Questionnaire (PHQ-9), 4) Patient-specific communication to primary care physician and 5) RMA measured patient status according to AAN ALS guidelines. Monthly and quarterly audits of performance were ascertained across 650 ± 188(SD) annual ALS encounters from 2009 through 2012.

Results: Cognitive-screening ($98.9 \pm 11.7\%$), psychiatric-screening (PHQ-2)($98.7 \pm 12.0\%$), psychiatric-follow-up (PHQ-9) ($98.4 \pm 17.4\%$), communications with primary care physician[$97.5 \pm 6.9\%$]assessments were performed according to practice standards achieving benchmark targets. RMA identified functional vital capacity (FVC) measurement as universal($100.0 \pm 0.0\%$)and the measure adjusted to assess the proportion of patients with FVC $< 60\%$ predicted offered respiratory support.

Conclusion: One of the initial five performance measures deployed in an ALS Clinic was regarded as auditable because it achieved the requirement of standard of practice. It was replaced by the RMA auditable measure that successfully evolved from a process-based performance measure to a patient-centered outcome measure. DSC is a clinic-based process for implementing performance measures to improve ALS clinic performance and is available internationally (5).

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C67 CASE MANAGEMENT AS AN ADJUNCT TO MULTIDISCIPLINARY CARE FOR ALS PATIENTS AND THEIR PRIMARY CAREGIVERS IN THE NETHERLANDS; NO EFFECT ON QUALITY OF LIFE OR CAREGIVER STRAIN

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Keywords: quality of life, quality of care, case management

Background: About 80% of the Dutch ALS patients and their primary caregivers are supported by one of the 43 multidisciplinary ALS care teams. From clinical practice we know that complex ALS care not always meets the needs of ALS patients and caregivers. Case management (CM) has been suggested as an innovative strategy to optimize care. Nevertheless, there is no evidence about the effectiveness of CM as an adjunct to usual care in ALS patients and their caregivers.

Objectives: The purpose of our study was to answer the following question: Does CM improve ALS patient's quality of life, caregiver's burden and perceived quality of care (QoC) of ALS patients and their caregivers?

Methods: We performed a cluster randomized controlled trial, with the ALS team as the unit of randomization. Participating ALS patients and caregivers received CM plus usual care or usual care only, conditional upon their team. Throughout 12 months, two occupational therapists provided CM and visited participants at home at study entry and every three months. Primary outcome measure was the ALS Assessment Questionnaire-40 items, domain Emotional Functioning (ALSAQ-40 EF). Secondary outcome measures were the Caregiver Strain Index (CSI) and QoC (rating score, range 0 to 10 = best possible). We performed assessments at baseline, four, eight and 12 months. We analysed change in emotional functioning and caregiver strain using a multilevel analysis. We used area under the curve analysis for the effect on perceived QoC.

Results: Thirty one teams recruited 71 patients and 66 caregivers for the intervention group and 61 patients and 60 caregivers for the control group. The extent to which participants relied on CM varied widely. Actions of the case manager were mostly in the area of emotional well-being, practical support and providing information. At baseline ALSAQ-40 EF was 19.6 (standard error (SE) 2.0) in both groups and did not change over time (0.56 (SE 0.57) /4 months; $p = 0.331$). In both groups, CSI scores increased from 5.3 (SE 0.4) at baseline with 0.7 (SE 0.1) points/4 months ($p < 0.0001$). We found no effect of CM on changes in emotional functioning or caregiver strain from baseline to 12 months. ALS patients from both groups rated their perceived QoC at baseline with a median score of 8 and caregivers with a median score for patient care of 8 and for caregiver care of 7.5. During follow-up, perceived QoC did not change and we found no significant effect of CM.

Discussion and conclusions: Our case management model as adjunct to multidisciplinary care had no effect on emotional functioning, caregiver strain and perceived QoC. One possible explanation is that multidisciplinary care in the Netherlands, which was high-ranked by the participants, leads to a high level of emotional functioning of ALS patients.

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C68 USE OF A COMPUTER-BASED DECISION AID CAN IMPROVE CLINICIAN UNDERSTANDING OF TREATMENT WISHES OF PATIENTS WITH ALS

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Keywords: advance directives, advance care planning, quality of life

Background: Patients with amyotrophic lateral sclerosis (ALS) face inevitable physical decline, necessitating reflection about, and decisions regarding, advance care planning (ACP).

Objectives: To determine whether a computer-based decision aid for ACP can help improve communication about end-of-life issues between patients with ALS and the clinicians who treat them.

Methods: Patients in an ALS multidisciplinary clinic were invited to use a computer-based decision aid to help them think about and document treatment preferences if they are unable to speak for themselves. Before patients used the decision aid, the ALS clinic team was presented with 3 hypothetical vignettes, and asked which particular treatments they would provide the patient in each scenario (ventilator, cardiopulmonary resuscitation, dialysis, feeding tube, etc.). The team also was asked how confident they were that they could "appropriately translate the patient's goals and values into clinical decisions that accurately reflect his or her wishes" (1 = not at all; 10 = extremely). Patients then used the decision aid, and generated an advance directive. The clinic team met three months later, reviewed the advance directive, and the vignette-based treatment decision process was repeated. Patients were then interviewed by telephone and asked which treatments they actually would want for each scenario. For each decision, concordance was determined between the patient's wishes and the ALS team's treatment plan. Summary scores were expressed as percent agreement, and pre-post comparisons were made using paired t-tests. Patient knowledge, anxiety, and self-determination were also compared pre and post intervention. The study was approved by Penn State's IRB.

Results: 44 patients participated (66% male, 77% married, mean age 58 years). At the time of enrollment, 50% had completed an advance directive. Prior to the intervention, concordance between patient wishes and the clinic team decisions was low (mean = 54.3%, SD 5 34.3). Following the intervention, concordance was dramatically higher (mean = 92.8%, SD 5 10.3; $p < 0.001$). ALS team member mean confidence increased significantly pre-post intervention (from 3.3 to 6.4; $p < 0.001$). Additionally, patient knowledge of advance care planning increased significantly after the intervention (from 47.8% correct responses to 66.3%; $p < 0.001$), without any adverse effect on patient anxiety or sense of self-determination.

Discussion and conclusions: If clinicians are to make medical treatment decisions that are consistent with patient wishes, they must have a good working knowledge of patients' values, goals and preferences, and the confidence to make the right choices. Use of a computer-based decision aid by patients can not only help the patients become better informed about ACP but can also improve the likelihood that the clinical team is knowledgeable about patient wishes, and confident in their ability to translate this into a medical plan. Clinicians caring for patients with ALS should consider integrating such decision support tools into their practice.

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C69 ALSPARTNER - INTERNET PLATFORM FOR COMPREHENSIVE ALS CARE

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Keywords: internet, home care, devices, aids

Background: People with ALS are dependent on provision of comprehensive care including assistive devices (mobility, transfer and advanced communication aids, orthetics) as well as physiotherapy, occupational therapy and speech therapy. Due to the severity and the progressive character of ALS there is an immense need for coordination.

Method: ALSPartner (AP) is a combined concept that unites social-medical service provision (case management), web-based technologies (www.ambulanzpartner.de) and an open network of ALS-trained home care provides. AP manages demanding organizational and care-related tasks liaising between outpatient departments, specialist medical practices, and specialized service providers that are coordinated, documented and visualized on a secure internet platform. The portal comprises a "secure personal care account" featuring all assistive devices and physical therapies, a status report on care provision processes and the option of rating all products and medical services.

Results: Between April 2011 and March 2012, 1040 patients were included - based at the ALS clinics at the Charité University Hospital in Berlin and of Ruhr University in Bochum. 3400 assistive devices and 620 physical therapies were coordinated. The pilot phase stretching a period of 12 months showed high acceptance of AP with a patient participation rate of 78% and a drop-out rate of less than 1%. Data on patient satisfaction are being captured from the perspective of different user groups. We demonstrate the meaningful use of electronic health record (EHR) in the user scenario of ALS.

Discussion: It is AP's prime intention and mission to bridge gaps and overcome barriers between professional groups, individuals playing different roles and separate care provision modules. The portal and the healthcare service provision structure enhance communication and cooperation between doctors, therapists and healthcare providers pertaining to various expert groups all acting in the ALS paradigm.

Acknowlewdgement: The project was supported by Stahlwerk Georgsmarienhütte Foundation, the Initiative 'Hilfe für ALS-kranke Menschen', the Air Berlin Fund for ALS Therapy Research, and funding of the German Ministry for Education and Research (BMBF) for the projects 'ServCare_ALS', and 'Innovationsdramaturgie nach dem Heldenprinzip'. TM and CM are founders of the internet platform AmbulanzPartner.

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C70 BULBAR MOTOR DETERIORATION IN ALS

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Keywords: bulbar deterioration, speech production, bulbar assessment

Background: Bulbar symptoms associated with ALS have a devastating effect on quality of life and significantly shorten survival. To date, surprisingly few programmatic research efforts have been directed toward understanding the natural history of bulbar symptoms. Major obstacles have been the inaccessibility and complexity of the speech apparatus. This investigation responds to this need by studying bulbar decline longitudinally and comprehensively using instrumentation-based analysis of speech behaviors. Longitudinal patterns of decline were investigated to identify sensitive quantitative indicators of the rate of bulbar deterioration, and to determine which speech subsystem measures accurately predict the onset of speech decline and the subsequent loss of oral communication.

Objectives: To determine (1) the sensitivity of multiple measures of bulbar function to disease progression, (2) the relations between speech system and subsystem measures, and (3) the degree of individual variation in speech subsystem impairment.

Methods: Fifty people with ALS were studied every three months for two years. Quantitative indices of motor deterioration were obtained for multiple speech subsystems including respiratory, phonatory, resonatory, and articulatory. To date, nine of the participants were diagnosed as bulbar-onset and eighteen as spinal-onset, four were diagnosed as both bulbar and spinal, and six were not specified. To ensure that the data sample included individuals who were experiencing bulbar decline, all participants will exhibit at least a 10% drop in speech intelligibility and/or a 20% drop in speaking rate. Recently developed three-dimensional motion-capture technologies were used to quantify longitudinal changes in lip, jaw, and tongue movements; aerodynamics were used to quantify declines in respiratory drive and resonatory function; and acoustics analyses were used to quantify declines phonatory function. Latent growth modeling was used to characterize individual and group patterns of deterioration in speech performance, and to establish associations between speech subsystem decline and speech loss.

Results: Of the speech subsystem measures, the velopharyngeal and oral articulatory measures exhibited a much faster rate of decline than did speech intelligibility. Despite differing levels of severity, participants showed a similar pattern in the speech subsystems that were most affected. Speech intelligibility was not correlated with speech subsystem measures.

Discussion: Findings to date suggest that speech subsystem measures decline more rapidly than speech system variables

The surprising lack of association between speech subsystem decline and speech intelligibility may be explained by prior reports suggesting that subsystem decline predates changes in speech intelligibility.

Conclusions: These findings suggest that speech subsystem measures of bulbar function can be identified prior to commonly used clinical measures of bulbar involvement such as speech intelligibility and speaking rate, and that these subsystem measures may provide sensitive outcome measures of bulbar involvement for clinical trials.

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C71 ALS MANAGEMENT AND SURVIVAL IN MODENA, ITALY: A STUDY ON A TEN-YEAR PROSPECTIVE POPULATION-BASED COHORT

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Keywords: survival, prognostic factors, therapeutic intervention

Objective: A number of clinical factors have been reported to predict ALS survival: age and site of onset, the severity and the rate of disease progression, the degree of diagnostic certainty, and the presence of dementia. Riluzole, enteral nutrition, non-invasive ventilation (NIV) and interdisciplinary care are also accompanied by a higher survival rate. We performed a study focused on ALS survival based on a population-based series, with particular attention to respiratory management and therapeutic intervention.

Methods: We registered all patients diagnosed with ALS between 2000 and 2009 and resident in Modena (population: 694,580). From 2000 onwards, a Centre for MND has been active in our province as well as a prospective registry collecting all incident cases. Demographic and clinical details were collected together with information about nutrition and ventilation support.

Results: Among the collected 193 incident cases, 47.67% underwent NIV. Patients who underwent NIV were younger. Phenotype did not influence the likelihood to undergo NIV. Patients followed by ALS multidisciplinary centres, as well as patients who underwent enteral nutrition had significantly higher probability to undergo NIV (OR 5.6 and 6.3 respectively). Forty-seven patients (24.35%) underwent tracheostomy (always after informed consent). Tracheostomised patients were younger (42.86% of patients $<$ 55yrs, 29.63% of patients aged 55–74yrs, 10.29% of patients $>$ 74yrs). There were no differences between genders and among phenotypes, except for bulbar ALS, who underwent tracheostomy significantly less frequent than other phenotypes. The presence of dementia or multidisciplinary approach did not influence the likelihood of being tracheostomised. The 49.22% of patients underwent to PEG. Patients who underwent to PEG presented more frequently with bulbar or classic phenotype, were younger and followed in multidisciplinary centre. The median survival time from onset to death was 41 months. The overall 3-year, and 5-year survival rates were 54.36%, and 28.81%, respectively. At univariate analysis, factors related to survival (from onset to death, $p < 0.05$) were: age at diagnosis, sex, phenotype (classic vs bulbar vs UMND vs flail vs respiratory phenotype: 32, 26, 67, 67, 18 months respectively), riluzole treatment (yes vs no: 43 vs 31 months), tracheostomy. Factors not related to survival were presence or absence of dementia, follow-up at an ALS centre, PEG or NIV. In the Cox multivariable model, the factors independently related to a longer survival were age ($p = 0.002$) and riluzole treatment ($p = 0.005$).

Discussion and conclusions: Surprisingly in our observational study, some procedures like PEG and NIV did not influence ALS survival. Also surprising are data about riluzole treatment which determines a gain in ALS survival of 12 months. This observational study describes the effect of our management and therapeutic intervention on ALS in a setting, which may approximate routine clinical practice more closely than RCT, but effects of uncontrolled potential confounders cannot be excluded.

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SESSION 10A ROLES OF NON-NEURONAL CELLS

C72 NEURON-ASTROCYTE CROSSTALK IN ALS

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C73 GENE EXPRESSION PROFILING OF ASTROCYTES FROM DIFFERENT DISEASE STAGES OF THE SOD1G93A MOUSE MODEL OF ALS REVEALS PERTURBATIONS IN LYSOSOMAL FUNCTION AND CHOLESTEROL METABOLISM

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Keywords: astrocytes, lysosomes, cholesterol

Background: Astrocytes play an important role in disease progression in the SOD1^{G93A} transgenic mouse model(1)and show a selective toxicity to motor neurons(2, 3)but the toxic factor(s) have not been identified. Laser-capture microdissection (LCM) allows individual cells to be isolated. We have previously published analyses of LCM motor neurons (MN) from the SOD1^{G93A} and the Vascular Endothelial Growth factor (VEGF) transgenic mouse models of ALS, which revealed altered carbohydrate and lipid metabolism in the SOD1^{G93A} model(4)and a downregulation of cholesterol l biosynthesis in the VEGF model(5). We have published data from LCM astrocytes from pre-symptomatic (60 day) SOD1^{G93A} mice, which revealed perturbed lactate metabolism and pro-NGF –p75 receptor signalling (6).

Methods: Astrocytes were isolated by LCM from spinal cord of symptomatic (90 day) and late-stage (120 day) timepoints from SOD1^{G93A} mice and non-transgenic littermates. cRNA was hybridised onto the Affymetrix Mouse Genome 430_2 Genechip and microarray analysis performed using Genespring GX (Agilent Technologies Inc) software with the probe logarithmic intensity error (PLIER) algorithm.

Results: 266 and 1834 genes were differentially expressed at the symptomatic and late disease stage. Annotation clustering analysis showed an upregulation of many genes in inflammatory pathways but also an upregulation of lysosomal genes (DAVID enrichment score 2.44) such as Cathepsin D (+2.32 and + 3.28 fold at 90 & 120 days) and Laptm5 (+2.81 & +5.7 at 90 & 120 days). In late-stage astrocytes there is a down regulation of multiple genes in cholesterol and steroid biosynthesis (hydroxysteroid 11-beta dehydrogenase 1 -2.32 & 5.05; hydroxysteroid (17-beta) dehydrogenase 7 -2.05 & 2.88) storage and excretion of cholesterol (24-dehydrocholesterol reductase −2.10 at 60 & −2.66 at 120 days) and uptake of cholesterol (low density lipoprotein receptor & lipoprotein lipase (−3.39 and −11.21 respectively). We are currently conducting further validation and functional assays.

Conclusions: We have found evidence for altered lysosomal function in SOD1^{G93A} astrocytes at symptomatic and late-stage disease. Cathepsin D is upregulated in SOD1^{G93A} mice spinal cord (*7*)but decreased in human mutant SOD1 MN(*8*), whilst the cathepsin inhibitor Cystatin C is decreased in CSF of ALS patients (*9, 10*). Furthermore, overexpression of Laptm5 increases lysosomal membrane permeabilisation, Cathepsin D leakage and non-caspase dependent cell death. Cholesterol levels are increased in the spinal cord of SOD1^{G93A} mouse model at pre-symptomatic and endstage as well as in spinal cord of patients with ALS (*11*). Excess cholesterol is toxic to MN(*12*)and altered cholesterol metabolism is seen in MN at end stage from the SOD1^{G93A} and the VEGF mice (*4, 5*). We believe that, like several other late onset neurodegenerative diseases, cholesterol transport abnormalities may contribute to motor neuron injury in ALS and that astrocytes play a key part in this process.

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C74 ASTROCYTES FROM FAMILIAL AND SPORADIC ALS PATIENTS ARE TOXIC TO MOTOR NEURONS

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Keywords: astrocytes, SOD1 gene therapy

Background: Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron (MN) disease with astrocytes implicated as a significant contributor to MN death in familial ALS (fALS). However, these conclusions, in part, derive from rodent models of fALS based upon dominant mutations within the superoxide dismutase 1 (SOD1) gene which account for less than 2% of all ALS cases. Studies performed in fALS mouse models have implicated non-neuronal cells such as microglia and astrocytes in the progression phase of fALS2. In particular, *in vitro* co-culture systems have shown that MNs perish in the presence of astrocytes harboring SOD1 mutations. However, all of these *in vitro* and *in vivo* studies have been conducted

using models that highly overexpress mutant SOD1, which may not fully mimic the actual disease.

Objectives: To determine whether astrocytes from ALS patients are also toxic to MN *in vitro* and determine the role of SOD1 in sporadic ALS (sALS) cases.

Methods: We generated astrocytes from post-mortem spinal cord tissue from both fALS and sALS patients. Briefly, between 24 and 72h after death, neural progenitor cells (NPCs) were isolated using Percoll gradient centrifugation. NPCs were differentiated into astrocytes, neurons and oligodendrocytes using different culture medium and growth factors. Astrocytes were obtained supplementing the culture medium with 10% FBS.

Human astrocytes were plated in 96 well plates coated with laminin at a density of 10,000 per well. Two days after, GFP positive motor neurons (MN) were sorted by FACS and cultured on top of the astrocytes at a density of 10,000 per well in MN media. After 24 hrs, cytosine arabinose was added for 48 hrs in order to eliminate any remaining dividing NPCs or embryonic stem cells. GFP positive neurons were counted on day one after plating, three and six (end of experiment).

SOD1 expression in astrocytes was knocked down by lentiviral transduction expressing siRNA sequences. In addition, lentiviruses were used to overexpress either human wild-type SOD1, SOD1 G93A or SOD1 A4V by the CMV promoter in astrocytes.

To evaluate the levels of SOD1 knockdown in sALS, SOD1 was analyzed by ELISA.

Results: Astrocytes derived from both patient groups (sALS and fALS) are similarly toxic to MNs. In addition, we show that SOD1 is a viable target for sALS, as its knockdown significantly attenuates astrocyte-mediated toxicity towards MNs.

Conclusions: Our data highlight astrocytes as a non-cell autonomous component in sALS and provide the first *in vitro* model system to investigate common disease mechanisms and evaluate potential therapies for sALS and fALS.

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C75 ASTROCYTES EXPRESSING THE HUMAN TDP43 A315T TRANSGENE ARE NOT TOXIC TO WILD-TYPE MOTOR NEURONS

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Keywords: TDP43, astrocyte, glia

Background: Recent studies have highlighted a role for not only neurons, but also glial cells in ALS pathogenesis. The most characterized ALS-linked gene is superoxide dismutase 1 (SOD1) and the majority of studies implicating glia have focused on transgenic mouse models of mutant SOD1 expression. Recently, mutations in the RNA-binding protein TDP43 have been linked to ALS and transgenic mice expressing human mutant $TDP43^{A315T}$ have been created. Although the mutant TDP43 is expressed in both neurons and glia in these mice, the contribution of $TDP43^{A315T}$-expressing glia to neuronal degeneration has not been investigated.

Objective: To determine whether astrocytes derived from the $TDP43^{A315T}$ mouse model cause damage to wild-type motor neurons (MNs).

Methods: Glial-restricted precursors (GRPs) were isolated from $TDP43^{A315T}$, $SOD1^{G93A}$, or wild-type (WT) littermate mice and 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 three months post-transplantation.

Results: To verify the astrocytes were expressing the TDP43 transgene, levels of human TDP43 were measured and comparable expression was seen in the GRP-derived astrocytes compared to neurons isolated from $TDP43^{A315T}$ mice. $TDP43^{A315T}$ astrocytes did exhibit increased cytoplasmic TDP43 mislocalization compared to WT littermates ($WT = 5.4+/-0.2$, $TDP43^{A315T} = 11.9+/-0.9$), however, no differences were noticed in astrocyte morphology or differentiation ability. Next, astrocytes were co-cultured with WT MNs and decreased MN survival was observed with $SOD1^{G93A}$ astrocytes, but not between WT or $TDP43^{A315T}$ astrocytes ($WT = 36.6+/-3.4$, $TDP43^{A315T} = 42.5+/-5.0$, $SOD1^{G93A} = 15.4+/-2.2$ percent of MNs surviving). To examine the effects of $TDP43^{A315T}$ astrocytes on WT MNs *in vivo*, WT, $TDP43^{A315T}$, and $SOD1^{G93A}$ GRPs were transplanted to the cervical spinal cord of WT rats. Rats receiving $SOD1^{G93A}$ GRP-derived 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 either WT or $TDP43^{A315T}$ astrocytes ($WT = 7.4+/-0.6$, $TDP43^{A315T} = 7.5+/-0.7$, $SOD1^{G93A} = 4.6+/-0.5$ MNs per ventral horn).

Conclusions: Our results show that $TDP43^{A315T}$ astrocytes from this mouse model do not possess the same MN toxicity *in vitro* or *in vivo* as has been shown for mutant SOD1-expressing astrocytes. Mutant TDP43 damage may occur solely within neurons or originate from other glial cell compartments besides astrocytes in this model, suggesting that astrocyte-derived MN damage may not be a shared pathway for all forms of ALS.

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C76 IMMUNE MODULATION AS A THERAPEUTIC STRATEGY FOR ALS

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Keywords: immune modulation, neuroinflammation, macrophage

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurological disorder where the precise mechanism of disease onset and progression remains unclear. However several studies have demonstrated there is a humoral immune response prior to the onset of severe clinical symptoms. Recently, elegant murine genetic studies, crossing $SOD1^{G93A}$ mice into either $RAG2^{(-/-)}$ or $CD4^{(-/-)}$ backgrounds, have provided evidence that infiltrating T cell populations are neuroprotective and not cytotoxic. We have recently reported that the costimulatory pathway is activated in spinal cord, skeletal muscle, and sciatic nerve during disease progression

in SOD1^{G93A} mice. We have recently shown that treatment of SOD1^{G93A} mice with a blocking antibody to CD40L (MR1) ameliorated several pathophysiological parameters associated with disease onset and progression. MR1 treatment improved survival, delayed disease progression, improved motor neuron survival, decreased astrocytosis, and decreased the accumulation of immune cells on peripheral nerves in SOD1^{G93A} mice.

Objectives: To extend these findings, we have examined other immune modulators that impact T cell subsets, as well as certain traditional immunosuppressive treatments, in an effort to understand the therapeutic mechanism of action of immunomodulatory treatments in SOD1G93A mice. We tested treatment strategies that have demonstrated efficacy in preclinical models of tissue transplant and autoimmunity, including compounds that have now been FDA approved for use in human autoimmune diseases. Here we report the impact of treatment with rapamycin, FK506, anti-CD3 T cell depleting antibody, anti-CD8 depleting antibody, anti-CD40L antibody, combined FK506/rapamycin, or fingolimod in the SOD1G93A animal model.

Methods: In order to test each of these compounds in SOD1^{G93A} mice pharmacokinetic (pk) studies were performed along with pharacodynamic (pd) studies to optimize dose selection. Based on the pk and pd studies chronic dosing experiments were conducted in SOD1^{G93A} mice to assess treatment impact on disease onset, progression, body weight maintenance, survival, and markers of inflammation in the periphery and central nervous systems.

Results and discussion: While some of these treatments ameliorated disease pathophysiology and improved survival (Fk506/rapamycin, fingolimod) others exacerbated disease (rapamycin). We will show how these compounds impact neuroinflammatory pathways in the central nervous system using whole genome microarray profiling. In addition to effects on central nervous system inflammation, we will also discuss how these treatments affect the regulation of peripheral lymphocyte populations and macrophage-mediated attack on peripheral nerves. Treatment of SOD1^{G93A} mice with either anti CD40L antibody or fingolimod have significant effects on reducing CD68 + macrophage accumulation on peripheral axons in skeletal muscle.

These studies are particularly important as we consider translating immunomodulatory strategies into potential clinical therapies for ALS patient. In addition, many of these immunomodulatory strategies may be utilized in conjunction with other potential treatments such as cell-based therapies for ALS.

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SESSION 10B RESPIRATORY SUPPORT

C77 MULTIDISCIPLINARY RESPIRATORY CARE IN ALS

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The respiratory complications of ALS cause intolerable symptoms and are the most frequent cause of death in ALS. Respiratory interventions such as non-invasive ventilation can significantly improve quality of life and survival. Therefore, respiratory evaluation, care and research deserve considerable attention in ALS centres worldwide.

The 2009 American Academy of Neurology Practice Parameter recommends the consideration of multidisciplinary clinic referral. Respiratory therapists but not pulmonary physicians are among the suggested clinic personnel. The practice parameter recommends assessing respiratory function with upright and supine spirometry, overnight pulse oximetry, maximal inspiratory pressures, cough peak flows and sniff nasal pressures. Based on the results of these tests, both non-invasive and invasive ventilation are recommended, as is assisted cough techniques. More recently additional respiratory interventions have been studied and proposed such as diaphragm-pacing systems. Proper utilization of these many tests and interventions can be complicated and may require collaboration between several clinical disciplines.

Many questions persist regarding respiratory management of ALS including how non-invasive ventilation should be initiated, monitored and titrated. Should it be started in the home, clinic, hospital or sleep lab? Where do sleep studies and the sleep lab fit into the care of the person with ALS? When and how often should the various pulmonary tests be used? When should non-invasive ventilation be implemented? When should tracheostomy be considered? When more than one pulmonary device is used, which combination of respiratory interventions is most effective?

Though effective treatments exist, there is evidence that people with ALS underutilize respiratory interventions. Also, much of the research and clinical efforts in ALS are directed at optimizing ventilation but secretion clearance is problematic and is ultimately the complication that proves to be untreatable and fatal.

Continued research efforts are needed to develop better respiratory interventions and optimize the delivery and utilization of current ones. It is imperative that all members of the ALS care team are aware of respiratory issues in ALS. This includes neurologists, advanced practice nurses, and physical therapists. Education about respiratory complications and treatments should be provided soon after the diagnosis is given in all people with ALS, regardless of their respiratory status. While many different personnel can and should be trained to assess and treat the pulmonary complications of ALS, it is this author's opinion that the most effective respiratory care is delivered in centres in which there is a pulmonary physician with expertise in ALS who works closely with the neurologists and a skilled, dedicated respiratory therapist. Respiratory interventions for ALS improve quality of life and survival but they need wider use with more thorough ongoing assessment and adjustment.

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C78 STILL BENEFICIAL AS MOTOR NEURONE DISEASE (MND) PROGRESSES? PROLONGED USE OF NON-INVASIVE VENTILATION (NIV) FROM MONTH 12 UNTIL DEATH

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Keywords: non-invasive ventilation, palliative care, quality of life

Background: Previous studies have reported on the positive impact of non-invasive ventilation (NIV) in motor neurone disease (MND). However, little has been studied about how patient perceptions of NIV may evolve at the later stages of the illness.

Objectives: The aim of this prospective study was to understand the experience of NIV at all stages of its use in MND.

Methods: This analysis is part of a bigger study of 35 patients followed every three months from time of assessment for NIV until death. 5/35 patients (female = 1, mean age = 62.8 years) met the criteria for this analysis, namely at least 12 months of NIV use (mean = 25 months; range = 20 to 32 months). Serial semi-structured interviews were available on patients' perceptions of NIV from month 12 until death. Interviews were transcribed verbatim and analysed using qualitative analysis.

Results: The data showed that there are two major concerns for MND patients: symptom management and terminal prognosis. Resilience, active problem solving attitude, adjustment, and pragmatic approach to equipment were the common attitudes towards ongoing symptom management. NIV benefits their daytime sleepiness, fatigue, breathing and speech. As the disease progressed, some of the benefits of NIV diminished (eg fatigue), yet patients reported increased perceived benefit for other symptoms (eg breathing). Regarding the terminal prognosis, two distinct approaches were observed; acceptance leading to contentment, and avoidance resulted from their anxiety. Whilst perceived benefit for symptom management changed with time, patients consistently valued NIV as life-prolonging treatment. Therefore, NIV was held to both prolong life and ameliorate symptoms, thereby enhancing life quality. Desire to engage with life was expressed by patients and NIV was perceived to significantly assist this, thus it was positively experienced and consistently used.

Discussion: The study shows that the true benefit of NIV at the later stages of MND is not restricted to the management of symptoms, but also impacts on psychological well-being. The use of NIV was experienced as positive for its psychological and symptomatic benefits and patients remained willing to engage with the treatment.

Conclusions: This study adds to the existing evidence based on the role of NIV in MND, by providing information on its life-enhancing benefits even at the terminal stages of the disease.

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C79 USING TRANSCUTANEOUS CARBON DIOXIDE MONITOR (TOSCA 500) TO DETECT RESPIRATORY FAILURE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: A VALIDATION STUDY

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Keywords: respiratory failure, TOSCA 500, carbon dioxide levels

Background: Hypoventilatory respiratory failure is the commonest cause of death in Amyotrophic Lateral Sclerosis (ALS). Early diagnosis of respiratory failure and treatment with Non-Invasive Ventilation (NIV) offers the best survival advantage currently available, as well as an improved quality of life. The UK National Institute for Health and Clinical Excellence and American Academy of Neurology recommend regular screening for respiratory failure, following a diagnosis of ALS. Symptom evaluation and respiratory function tests are used to screen the patients for respiratory failure ($PCO_2 > 6$ kPa by definition). Currently there is no single test of respiratory muscle strength which can predict hypercapnia with high sensitivity and specificity. Moreover, volitional tests of respiratory function have serious limitations in patients with severe bulbar weakness. Transcutaneous carbon dioxide monitoring is a non-invasive method of measuring arterial carbon dioxide levels enabling simple and efficient screening for respiratory failure.

Objectives: The aim of this study is to validate the accuracy of carbon dioxide level recorded transcutaneously with a TOSCA *500* monitor in patients with ALS.

Methods: This is a prospective, observational study of 40 consecutive patients with ALS. The partial pressure of carbon dioxide in each patient was determined by both transcutaneous monitoring and by an arterialised ear lobe capillary blood sample ($PtcCO_2$ vs. $PaCO_2$). The carbon dioxide levels obtained with these two methods were compared by Bland-Altman analysis.

Results: The mean difference (bias) between the two measurements was -0.08 kPa, with a standard deviation (SD) of 0.318 and standard error of mean (SEM) of 0.05. Pearson's correlation coefficient of 0.808 showed a statistically significant relationship between the two methods ($p < 0.001$), but not that they necessarily agree. The Bland-Altman plot showed overall good agreement between the two measurements with 95% limits of agreement (bias $\pm$ 1.96SD) between 0.553 and -0.719 kPa. The difference was < 0.5 kPa in 90% of the recordings. Four of the forty measurements had a difference of > 0.5 kPa, with a maximum recorded difference of 0.95 kPa. In 22 patients $PtcCO_2$ reading was higher than $PaCO_2$ but no consistent numerical relationship was identified between the two measurements and hence an application of a correction factor cannot be recommended.

Discussion and conclusions: TOSCA *500* is a useful device to be utilised for the assessment of ALS patients, enabling regular and non-invasive screening for respiratory failure. It is necessary to consider the $PtcCO_2$ readings in the wider clinical context, especially if the readings are not compatible with the symptoms or other tests of respiratory function (e.g., forced vital capacity). We recommend that a $PtcCO_2$ reading of > 6.0 kPa be verified by an arterial blood gas analysis, so that the decision for intervention with ventilatory support is planned without any ambiguity.

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C80 DECISION-MAKING ABOUT NON-INVASIVE VENTILATION (NIV) AND GASTROSTOMY IN ALS: RELATIONSHIP BETWEEN EARLY ATTITUDES TO TREATMENT AND DECISIONS MADE

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Keywords: decision-making, non-invasive ventilation, gastrostomy

Background: Non-UK prospective studies have estimated non-invasive ventilation (NIV) and gastrostomy use ranging between 4–30%. A recent UK survey reported increased NIV use. Little data exist on incidence of gastrostomy in England and Wales. Researchers have started to describe patients' attitudes towards these interventions prior to needing them, finding that early treatment preferences for interventions strongly predict actual treatment choice.

Objectives: This prospective, population-based study aimed to identify factors influencing decision-making about NIV and gastrostomy in ALS. Here, we describe our sample's attitudes to interventions at study baseline, other baseline characteristics and their relationship to subsequent decisions.

Methods: Seventy-eight people with ALS were recruited from the South East ALS Register. At study enrolment, none had made a clinical decision about NIV or gastrostomy. In addition to completing several physical, cognitive, psychological and health service use measures, participants were interviewed about interventions they might subsequently be offered. Responses regarding NIV and gastrostomy were

coded according to seven themes: three 'passive' ('no mention of intervention', 'aware of intervention but reluctant to think about it', 'passive approach') and four 'active' ('keen to find out more', 'intervention considered, no decision made' and 'decision made to accept'/'decline'). Actual decision-making was monitored at three monthly intervals.

Results: The sample had a mean age of 62.5 years (SD 11.7), mean ALSFRS-R score of 35.3 (SD 7.5) and mean time since diagnosis of 12.5 months at recruitment. Forty-nine were male. Most had sporadic ALS (71) and non-bulbar onset.

The largest proportion of participants made no mention of gastrostomy (42.3%) or NIV (47.4%). Eighteen percent and 20.5% indicated awareness of gastrostomy and NIV, respectively, but were reluctant to think about them in advance. Two (2.6%) expressed a passive decision-making approach, believing healthcare professionals should make such decisions. A firm decision at baseline seemed more likely to have been made about gastrostomy (19.2%) than NIV (10.3%). More had made a decision to refuse gastrostomy (11.5%) than NIV (3.8%). Participants with familial ALS were reluctant to consider interventions or made no mention of them. Bulbar onset patients seemed more likely to have considered or made a decision about gastrostomy (8) than NIV (3).

Twenty-one gastrostomy (15 accepted; six refused) and 21 NIV decisions (19 accepted; two refused) were made by 32 participants (41%). Three out of four people who reported making a decision in advance about accepting gastrostomy were consistent with their earlier preferences. One of the three who made a baseline decision to accept NIV subsequently refused it.

Most first decisions for participants with bulbar onset concerned gastrostomy (89%); for those with non-bulbar onset, the majority were NIV decisions (52%). NIV decisions were taken closer to end-of-life (mean 2.7 months prior to death) than gastrostomy decisions (mean 6.1 months).

Discussion and conclusions: Results provide information on patients' decision-making across the disease, including attitudes towards NIV and gastrostomy before a clinical need for them arises. Nineteen percent of patients accepted gastrostomy (largely consistent with previous reports), while NIV use (24%) was slightly higher than previously reported. Gastrostomy was generally the first decision made, while NIV tended to be offered later in the disease when people were more unwell. Despite insufficient statistical power for formal testing, unlike previous studies, our findings suggest that early preferences for gastrostomy and NIV do not always predict subsequent treatment choices.

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C81 SHOULD RADIOLOGIC EVALUATION OF THE CHEST AND DIAPHRAGM BE ROUTINE PRACTICE IN ALS/MND PATIENTS?

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Keywords: diaphragm dysfunction, radiology, diaphragm pacing

Background: Patients with ALS/MND suffer from significant diaphragm dysfunction that leads to hypoventilation with subsequent respiratory failure. Standard assessment of respiratory function involves pulmonary function tests but these tests may underestimate unilateral diaphragm dysfunction. There is little literature on radiographic analysis of diaphragm function in ALS/MND patients.

Objective: Review chest radiologic evaluations to assess diaphragm abnormalities in patients with ALS/MND.

Methods: All patients with ALS/MND who had chest radiographic examinations were reviewed at a single site. All of the patients were under IRB approved protocols to assess for suitability of diaphragm pacing. All data were obtained prospectively although these were re-analyzed retrospectively with specific attention to diaphragm abnormalities. Chest x-rays were evaluated and assessed for right compared to left diaphragm differences using the standard values that the left diaphragm should be 1.5 to 2cm lower than the right diaphragm. Digital fluoroscopy was performed with a grid measuring system. Normal excursions would be expected to be 4–5 cm of movement. In this ALS population it was considered abnormal if the excursions were less than 3cm of movement. There were two sources of patients: prospective, nonrandomized, controlled, interventional trials under IRB and/or FDA approval for use of diaphragm pacing and standard of care use of diaphragm pacing following FDA-approved criteria.

Results: 111 ALS subjects had prospective radiographic evaluations available for review. On plain chest radiograph 65% of the subjects had an abnormality of the diaphragm noted. In 86% of these patients the initial report was noted as normal because radiologic interpretation focused on lung parenchyma but not diaphragm abnormalities. When films were re-assessed to evaluate the diaphragm, the report changed. On these radiographs a unilateral diaphragm abnormality was noted in 70% of the patients. Under digital real time fluoroscopy, 89% of the subjects had abnormal diaphragm movement during volitional inspiration.

Conclusion: Chest radiography can help diagnose diaphragm abnormalities in ALS/MND patients which can change therapy for patients. Significant diaphragm elevations independent of forced vital capacity demands therapeutic manoeuvres that include: changes in sleep position to maximize ventilation, use of non-invasive ventilation to prevent recumbent pulmonary atelectasis, and diaphragm pacing if the involved diaphragm is stimulatable. In a severely elevated diaphragm with paradoxical movement, although not studied yet in ALS, minimally invasive hemi-diaphragm implication could improve dyspnea scores as it does in idiopathic unilateral diaphragm paralysis. Future studies with spiral CT scans or dynamic MRIs can assess which part of the diaphragm that is involved. If the crucial posterior diaphragm is specifically involved then diaphragm pacing which can focus on this posterior diaphragm can help improve posterior lobe ventilation decreasing the risk of pneumonia. A routine chest radiograph requesting diaphragm analysis can help identify abnormalities and allow early recommendations for therapeutic interventions.

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SESSION 11A MURINE MODELS

C82 WHAT MAKES A GOOD ANIMAL MODEL?

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Animal models have been instrumental for identifying pathogenic mechanisms, target validation and evaluation of potential therapies for human diseases, including ALS/MND. Over the past two decades, animal models based on causative genes identified in familial ALS have been developed in efforts to advance our understanding of the pathogenesis of ALS and test therapeutic strategies to treat this devastating illness. Drawing on several animal models of MND (SOD1, DCTN1 and TDP-43) I will highlight the important lessons learned and address factors that contribute to a good animal model. This presentation will emphasize on recent advances in rodent models of TDP-43.

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C83 NEW ANIMAL MODELS OF ALS AND ALS/DEMENTIA

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Keywords: UBQLN2, OPTN, transgenic

Amyotrophic lateral sclerosis (ALS) is a paralytic and usually fatal disorder caused by degeneration of motor neurons in the brain and spinal cord, leading to respiratory failure and death. Currently, there is no effective treatment for ALS. Numerous clinical trials have been carried out, but have failed to show promising results. Development of effective therapies for ALS is largely hindered by limited knowledge about its pathogenic mechanism. Understanding the pathogenic mechanism, therefore, represents the major challenge to the ALS research community. Genetic and animal model studies in the past two decades have played the major role in providing the missing pieces of the mechanistic puzzle. However, due to the diversity of the disease causes, the convergent pathways to motor neuron degeneration in ALS remain elusive. Generation and integration of the data from different ALS models may provide important mechanistic insight into the pathogenic pathways that can be exploited for therapeutic intervention.

Mutations in OPTN, UBQLN2 and C9ORF72 have recently been identified as causes for ALS and ALS/dementia. But the molecular bases are not understood. UBQLN2 encodes an ubiquitin-like protein, ubiquilin2, which is involved in ubiquitinated protein degradation. Mutant UBQLN2 has been shown to impair the ubiquitin-proteasome system (UPS) and autophagy in cellular models, suggesting that impairment of the UPS and autophagy may underlie the pathogenesis of ALS. Importantly, the distribution of ubiquilinopathy in the central nervous system of ALS and ALS/dementia cases has been shown to be well correlated with motor and cognitive symptoms.

To explore the pathogenic mechanism, we have developed new transgenic mice overexpressing UBQLN2^{P497H}, OPTN^{E478G} or C9ORF72 with GGGGCC expansion.

The UBQLN2^{P497H} transgenic mice did not show obvious motor abnormality, but developed behavioral abnormalities and ubiquilinopathy in the central nervous system, especially the hippocampus, thus recapitulating some key clinical and pathological features of dementia observed in the human patients. We observed co-localization of ubiquilin2 and proteasome subunits, as well as OPTN in the inclusions, which were predominantly distributed in the dendritic spines. Mutant UBQLN2 impaired ubiquitinated protein degradation and led to a conversion of long-term potentiation (LTP) to long-term depression (LTD) in the transgenic mice. Thus, our data provide robust *in vivo* evidence that links impaired protein degradation to protein aggregation, dendritic spinal pathology, neurophysiological defects and neurodegeneration. The presence of OPTN in the hippocampal inclusions in the UBQLN2^{P497H} transgenic mice may imply a convergent role of UBQLN2 and OPTN in the UPS and autophagy defects in ALS. Data regarding the OPTN^{E478G} and C9ORF72 transgenic mice will be updated and discussed.

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C84 GENERATION AND CHARACTERIZATION OF NEW TRANSGENIC MICE TO INVESTIGATE THE ROLE OF FUS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FUS, transgenic mice, motor impairment

Background: About 3 % of ALS cases are caused by mutations in *FUS* (Fused in sarcoma) and the neuropathology of those cases is characterized by abnormal accumulation of FUS into neuronal and glial cytoplasmic inclusions. FUS is a multifunctional RNA/DNA binding protein, predominantly nuclear, but shuttling between the nucleus and the cytosol. In cell culture, mutant FUS accumulates in the cytoplasm as *FUS* mutations disrupt its nuclear localization signal leading to impaired nuclear import. However, the mechanisms of FUS-associated cell death remain unsolved.

Objectives: The aim of the project is to investigate the consequences of wildtype or mutant FUS overexpression *in vivo*.

Methods: Transgenic mouse lines expressing either human wild-type FUS (hFUSwt) or the truncated protein FUS-R495X (hFUSR495X) associated with aggressive familial ALS under the control of the prion protein promoter were generated by oocyte injection. Transgene expression was investigated at RNA and protein level. Survival, body weight, general welfare and motor performance with a modified SHIRPA test were monitored. Immunohistochemistry was performed at relevant time-points to investigate the presence of inclusion bodies, neurodegeneration and reactive gliosis.

Results: Total FUS expression in our transgenic mice is up to six fold compared to endogenous FUS in non-transgenic mice. Consistent with the *in vitro* data, hFUSwt is predominantly expressed in the nucleus as the endogenous protein, whilst hFUSR495X is massively redistributed to the cytoplasm in brain and spinal cord sections from our transgenic lines, without concomitant mislocalization of EWS and TAF15, as observed in post-mortem material from ALS cases with *FUS* mutations. Notably, the highest expressing hFUSR495X line showed reduced body weight, progressive decline of motor performance and premature death (median 81.5days for females; 112days for males). Histological analysis revealed vacuolated spinal cord motor neurons even at early ages. However, comparing the motor function of mice with similar expression of either hFUSwt or hFUSR495X (˜30% more than nontransgenic) suggests that hFUSwt is more toxic than the mutant. Furthermore, we observed that the expression of either hFUSwt or hFUSR495X leads to downregulation of the endogenous protein, indicating a similar autoregulation mechanism for FUS as recently described for the functionally related protein TDP43.

Discussion and conclusions: Our novel FUS transgenic mice will be valuable tools to elucidate the role of FUS and FUS mutations *in vivo* and to investigate its potentially distinct role in different cell compartments, including the highly debated issue as to whether the toxicity of the mutated protein arises from a gain-of-function or a loss-of-function mechanism. Our preliminary data suggest that FUS overexpression is a potentially harmful event, being the wt protein more toxic than the truncation mutant when expressed at similar level in transgenic mice. However, high expression of the mutant protein leads to premature death in transgenic mice.

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C85 CELL AUTONOMOUS MOTOR NEURON DEGENERATION CAUSED BY ALS MUTANT FUS: A NOVEL MOUSE MODEL OF DISEASE

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Keywords: FUS, mouse, model

Background: Although Amyotrophic Lateral Sclerosis (ALS) is largely a sporadic disease, research has focused on heritable forms of the disorder because clinical and pathological evidence suggests common pathogenic mechanisms. Mutations in the gene FUS (or TLS) were recently reported in rare ALS families, and FUS pathology has since been found in sporadic ALS, suggesting that FUS may provide a link between familial and sporadic disease mechanisms. Physical and functional interactions between FUS and TDP-43 – another RNA/DNA-binding protein involved in the pathogenesis of sporadic and familial ALS - have also led to speculation that the molecular pathways regulated by both of these factors are critical to our understanding of common disease mechanisms. How mutations in FUS cause ALS is unknown, but dominant inheritance of FUS mutations together with the finding of abnormal FUS-containing inclusions in degenerating neurons suggests a novel gain of function that is selectively toxic to motor neurons. However, it is also possible that mutant FUS acts as a dominant negative, inhibiting the normal activity of wild type protein. Whether mutant FUS causes motor neuron degeneration by a cell autonomous mechanism or – as is the case with mutant SOD1 – non cell autonomous effects contribute to the disease is also not known.

Objective and methods: To model the effects of ALS mutant FUS on motor neurons *in vivo*, we generated a conditional / CRE-dependent allele targeted to the mouse MAPT (tau) locus with which to overexpress mutant or wild type human FUS in specific cell types in the nervous system.

Results and discussion: Selective expression of ALS-associated R521C mutant FUS in motor neurons resulted in progressive motor neuron loss and associated denervation of limb muscles. Other hallmarks of FUS-mediated ALS, including cytoplasmic mislocalization and nuclear exclusion of FUS, as well as the formation of nuclear and cytoplasmic aggregates were also evident in these conditional mutants. The toxicity of mutant FUS was not observed when wild type human FUS was expressed from the same locus at comparable levels, demonstrating a dependence of the motor neuron phenotype on the ALS-associated mutation. These studies demonstrate that ALS mutant FUS causes cell autonomous changes in motor neurons that reproduce several pathological features of the disease. We present our initial characterization of a novel animal model of ALS.

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C86 DEGENERATION OF MOTONEURONS IN SOD1 MICE: THE SIZE HYPOTHESIS

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Keywords: morphology, 2 photon imaging, excitotoxicity

Background: Excitotoxicity has been proposed as a mechanism leading to the selective death of motoneurons in Amyotrophic Lateral Sclerosis (ALS). It is indeed known that increase excitation and hyperexcitability can cause the death of motoneurons. However, it has yet to be demonstrated that spinal motoneurons exhibit changes in their excitability in the days preceding their degeneration.

Objectives: The aim of this work is therefore to test whether spinal motoneurons show signs of hyperexcitability at ages just preceding the first denervations of motor units.

Methods: To test this hypothesis requires recording the intrinsic properties of motoneurons in SOD1 mice at an adult age. We therefore have developed two new preparations to

allow intracellular recordings of adult mouse motoneurons *in vitro* and in vivo, at an age (P50) just preceding the first denervations of motor units.

Results: Thanks to these two new preparations, we were able for the first time to compare the electrophysiological properties of adult motoneurons between SOD1 mice and WT animals. At odds with the excitotoxicity hypothesis we observed that SOD1 motoneurons did not exhibit signs of hyperexcitability in either region of the spinal cord, lumbar or sacrocaudal. For example, in the lumbar cord, neither the onset current for repetitive firing (4.9 ± 3.2nA; $N = 25$ vs. 6.2 ± 3.0nA; $N = 22$), nor the gain of the current-frequency relationship (12 ± 2Hz/nA; $N = 13$ vs. 16 ± 8Hz/nA; $N = 11$) were statistically different between SOD1 and WT mice. On the other hand, we observed that the input conductance of SOD1 motoneurons was significantly increased compared to controls. In the lumbar cord, the input conductance of motoneurons was 0.31 ± 0.13μS ($N = 25$) in WT mice, whereas it was 0.46 ± 0.18μS ($N = 25$; $p < 0.001$) in mutant animals.

This increase in conductance suggests that the size of the motoneurons could be increasing during the disease. We therefore measured the soma size and the number of primary dendrites by retrogradely labeling sacro-caudal motoneurons at P30 then observing slices of live spinal cord under two-photon microscopy. We observed that indeed, the soma volume was larger in SOD1 than in WT ($18183 \pm 17178\mu m^2$; $N = 36$ vs. $10592 \pm 8210\mu m^2$; $N = 31$; $p < 0.05$) and they also had more primary dendrites (6.8 ± 2.6; $N = 36$ vs. 4.6 ± 1.7; $N = 31$; $p < 0.0001$).

Discussion: Our new results show that motoneuron conductance continues to increase as the SOD1 animal matures, pushing some cells well above the normal limits. Yet, net excitability continues to remain normal, clearly requiring an increase in voltage sensitive currents as a compensatory mechanism. Thus motoneurons in ALS do not become hyperexcitable but instead undergo a notably successful homeostatic regulation for excitability. Although initially this homeostasis helps the animal maintain normal motor output, the progressive increases in cell size and density of membrane channels could lead to their degeneration. This is what we call the "size hypothesis".

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C87 EXCITOTOXICITY AND NEUROMUSCULAR JUNCTION DEGENERATION FOLLOWING SITE-SPECIFIC EXCITOTOXIN EXPOSURE IN VIVO

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Keywords: excitotoxicity, neuromuscular junction, axonal degeneration

Background: 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 by using site-specific excitotoxic insults, via osmotic minipumps, to the spinal cord and muscle.

Methods: To achieve site-specific excitoxotixity osmotic mini pumps (Alzet, model 1004), delivered a constant chronic infusion to either the L3-4 lumbar region of the spinal cord or the gastrocnemius muscle in the hind limb. A constant and chronic infusion of Kanic acid (KA, 1–5mM, 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 (5mM 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 L3-5 and muscle fibres of the gastrocnemius muscle, indicating 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 the number of motor neurons at 28 DPS in the 5 mM KA treated mice in comparison to 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 to control. Additional, in the YFP mice, analysis demonstrated that whilst there was a reduction in the number of synapses in the KA muscle there was also an increase in the number of branch points in each junction, indicating that whilst degeneration of the NMJ was present, NMJ remodelling was also occurring in the KA-treated mice. This compensatory plasticity may be an early event in the pathogenesis of axonal degeneration in ALS.

Conclusion: 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.

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C88 CONFOCAL MICROENDOSCOPY OF NEUROMUSCULAR JUNCTIONS DURING ACTIVITY-DEPENDENT SLOW SYNAPTIC DEGENERATION IN MICE

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Keywords: neuromuscular junction, microendoscopy, activity

Accumulating evidence suggests that degeneration of motor neurones in at least some forms of ALS begins at neuromuscular junctions (NMJ). We are therefore addressing two challenges: first, to protect motor neurones from degeneration by seeking ways to inhibit degeneration of their NMJ; and second, to promote compensation by surviving motor neurones and to sustain or boost their function at NMJ. We are

taking advantage in these studies of mice expressing the neuroprotective Wallerian degeneration-Slow (*Wld*^{S}) chimeric gene. The degeneration of axotomised motor nerve terminals in *Wld*^{S} mice occurs about 10 times more slowly than in wild-type mice. It is possible to monitor this protracted time course with confocal microendoscopy, using a 1.5 mm diameter optical fibre probe to image NMJ in transgenic t*hy1.2-YFP*/*Wld*^{S} double-homozygous mice. We are also screening other potential fluorochromes that might be useful for safely visualising synapses at unlabelled NMJ's. We have found that synaptic degeneration at axotomised NMJ in *Wld*^{S} mice is strongly sensitive to activity. Paralysis of NMJ in *Wld*^{S} mice by a preconditioning tetrodotoxin (TTX)-induced nerve conduction block for one week virtually abolishes the protective effect of *Wld*^{S} gene expression on motor nerve terminals. Conventional microelectrode recording five days after axotomy showed about a five-fold reduction in the number of muscle fibres responding with endplate potentials to nerve stimulation compared to those treated with saline prior to axotomy, or no previous surgery (TTX: $8.57 \pm 2.51\%$ of fibres; saline controls: $55.56 \pm 5.88\%$; nerve section only controls: $43.34 \pm 10.11\%$; Mean $\pm$ SEM; $n = 7, 3, 6$ mice, respectively; $p < 0.05$ ANOVA). Confocal microendoscopy revealed that the protective effect of *Wld*^{S} on axons in the tibial nerve remained unaltered by sciatic nerve block and muscle paralysis. Enhancing activity through voluntary aerobic exercise (through provision of running wheels) for one month prior to an axotomy challenge had no discernible effect on neuromuscular synaptic degeneration. However, enhancing activity in this fashion liminally promoted a more rapid recovery by compensatory nerve sprouting in partially denervated lumbrical muscles of wild-type mice. Together, the data constitute direct evidence that activity can modulate neuromuscular synaptic degeneration. Specifically, disuse increases the vulnerability of motor nerve terminals to neurodegenerative stimuli. We are presently combining *in vivo* confocal microendoscopic imaging with conventional electromyographic recording, to monitor the effects of activity on synaptic degeneration in longitudinal study; and, in parallel, developing an *ex vivo* assay to evaluate potential mechanisms of activity-dependent modulation of synaptic degeneration.

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SESSION 11B SURROGATE MARKERS

C89 THE UNIQUE METABOLIC PATTERN OF GLUCOSE METABOLISM IN EARLY C9ORF72-RELATED ALS: A FDG PET STUDY

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Keywords: PET, C9ORF72, neuroimaging

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative diseases characterized by both upper and lower motor neuron lesions. Recently a hexanucleotide repeat expansion in the first intron of *C9ORF72* gene has been reported to be the cause about 10% of cases. Data about glucose metabolic activity evaluated with positron emission tomography (PET) of these patients are still lacking.

Aim: The aim of this study was to assess by PET the distribution of [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG) in a series of ALS patients with *C9ORF72* hexanucleotide repeat expansion compared to normal controls and ALS patients with no mutations of ALS-related genes.

Methods: The ^{18}F-FDG PET/CT scans of 10 ALS patients carrying the *C9ORF72* mutation were compared to those of 30 ALS patients without genetic mutations, matched by age, gender, disease duration and site of onset. All patients performed PET within two months after diagnosis. Six ALS patients with *C9ORF72* mutation (60%) and 10 ALS patients without genetic mutations (33.3%) had FTD ($p = n.s.$). The control group included 40 subjects negative for neurological or neoplastic diseases. Differences were analyzed by statistical parametric mapping (SPM2) introducing age, gender and disease type (upper or lower motor neuron) as nuisance variables. SPM t-maps were thresholded at $p < 0.05$, corrected for multiple comparisons with the False Discovery Rate (FDR) option at voxel level.

Results: ALS patients with *C9ORF72* hexanucleotide repeat expansion and those without genetic mutations were comparable in term of age, gender and disease duration. Highly significant hypometabolism was found in *C9ORF72* as compared to ALS without genetic mutations in thalamus, anterior and posterior cingulate cortex, and medial frontal cortex (BA8) bilaterally, as well as in right prefrontal cortex (BAs 9,45), insula and caudate head. Significant hypermetabolism was found in C9ORF72 in bilateral cerebellum, in midbrain and in left claustrum, globus pallidus and putamen. Compared to controls, patients with C9ORF72 mutation had a relative hypometabolism in bilateral frontal cortex (BAs 6, 9, 10, 11, 45 and 46), bilateral caudate head and thalamus, midbrain and anterior cingulate cortex (BAs 32) and a relative hypermetabolism in midbrain. This alteration reflects the diffuse pathology and complex phenotype associated with *C9ORF72* mutations and may be specific of this mutation.

Conclusions: We have found that ALS patients with *C9ORF72* mutation are characterized by a pattern of glucose metabolism different from normal subjects and ALS patients without genetic mutation, with a relative hypometabolism of basal ganglia and anterior cingulate cortex and a relative hypermetabolism of midbrain and bilateral cerebellum. These alterations are consistent with the peculiar symptom constellation of patients carrying the *C9ORF72* hexanucleotide repeat expansion, encompassing ALS and FTD but also psychotic-like and exrapyramidal symptoms.

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C90 ASSESSMENT OF NEUROINFLAMMATION IN ALS WITH 18F-DPA-714 PET

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Keywords: microglia, DPA714, TEP imaging

Rationale: There is growing evidence of activated microglia and inflammatory processes in cerebral cortex in ALS. Activated microglia is characterized by an increased expression of the 18 kD atranslocator protein (TSPO). TSPO, highly expressed in phagocytic inflammatory cells tissue, is part of a protein complex associated with the outer mitochondrial membrane of many cells. TSPO, found in peripheral organs, is also expressed in the brain (astrocytes and microglia) and may be a useful biomarker for inflammation. Among TSPO radioligands for molecular imaging, the fluoro-ethoxy analog DPA-714 labeled with fluorine-18 has been developed. In this study, we evaluated the degree of neuroinflammation in ALS patients using PET with ^{18}F-DPA-714.

Methods: Ten ALS patients (6 bulbar and 4 spinal ALS) right-handed, who fulfilled the criteria of probable or definite ALS according to the Airlie House meeting (Brooks, 2000), without dementia according to the scores of the MMSE and the FAB tests, and naïve of riluzole and medications that might bias the binding on TSPO at the time of the inclusion

were prospectively enrolled as were eight healthy controls matched for age.

A 90 min cerebral dynamic acquisition was performed followed the injection of ^{18}F-DPA-714 (264 ± 59 MBq). Relative volume of distribution (DVR) were calculated for frontal, primary motor, supplementary motor, temporal, occipital and cerebellum cortex, thalamus and stem using ROI as defined in the MNI-AAL atlas and the Logan graphical method analysis with reference regions individually defined by cluster analysis. Comparisons between patients and controls were done using Student's *t* test.

Results: Significant increase of microglial activation was found in primary motor, superior motor and temporal ROI (t-test, $p = 0.017$, $p = 0.002$ and $p = 0.005$ respectively).

Conclusion: Although these results need to be confirmed on a larger sample, this strongly suggests that microglial activation is increased in ALS patients since the early stages of the disease. The ability to assess *in vivo* microglia activation might improve our understanding of mechanisms leading to occurrence of neuroinflammation in ALS and other neurodegenerative disorders and allow monitoring therapeutics efficiency. The presence of extra motor microglia activation might corroborate theories supporting that ALS should not be a prototypic motor neuron condition and that a continuum between ALS and FLTD can be drawn.

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C91 ATROPHY MEASUREMENT AS A DISEASE PROGRESSION BIOMARKER IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: spinal cord, atrophy measurement, progression biomarker

Introduction: Atrophy of the spinal cord is one of the neuroimaging features associated with amyotrophic lateral sclerosis (ALS) (1)(2). In a recent MRI study we have shown that cervical cord atrophy is associated with lower motor neuron degeneration and is correlated with clinical deficits (2). Whether atrophy measurement is a relevant biomarker for disease progression biomarker remains unknown.

Objectives: To longitudinally assess by MRI the atrophy progression at cervical and thoracic levels of the spinal cord in ALS patients and to test its relationship with the functional impairment changes as assessed by the revised ALS functional scale (ALSFRS-R).

Methods: Among the 29 ALS patients who had a spinal MRI in our first study, 14 patients (4 Females/10 males) underwent a second MRI after a mean follow-up of 11 months. At baseline, the mean age was 51 ± 11 years and the mean disease duration was 29 ± 28 months and the mean ALSFRS-R was 38.9 ± 6.1. Functional impairment in the upper limbs was assessed by the ALSFRS-R subscale considering the upper limbs items. At time of the second MRI, the mean upper limb ALSFRS-R was 2.9 ± 2.9 (versus 5.7 ± 2.3 at baseline, -48.8%). Subjects were scanned using a 3T MRI system (Tim Trio, Siemens Healthcare). Imaging parameters were: T2-weighted 3D turbo spin echo (52 sagittal slices, FOV = 280mm, TR/TE = 1500/120ms, voxel size = 0.9x0.9x0.9mm^3) (3). Cord sectional area was measured from the T2-weighted image at the vertebral levels from C2 to T6 using a semi-automatic method (4).

Results: Wilcoxon signed rank test showed a significant decrease over time in mean cord area (-2.8%, $p = 9\text{x}10^{-4}$). Spearman's coefficient showed significant correlation between mean cord sectional area changes and the ALSFRS-R upper limb changes ($r = 0.83$, $p = 2\times10^{-4}$).

Conclusion: This study suggests that atrophy is associated with functional changes over time. Although further studies in a larger population of ALS patients are needed, our result suggests that atrophy measurement may potentially be used as a disease progression biomarker in ALS.

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C92 THE EARLIEST CHANGES IN MOTOR UNIT PHYSIOLOGY IN ALS

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Keywords: electromyography, fasciculation potentials, disease progression

Introduction: There is little information on the earliest changes in motor unit (MU) physiology in ALS and the development of the classical neurophysiological features of ALS over time. It is believed that fasciculation potentials (FPs) and abnormal MU potentials (MUPs) are early features and that fibrillations and sharp-waves (fibs-sw) appear later, but whether this is an orderly relationship is largely unknown.

Methods: We studied the tibialis muscle (TA) in three groups of subjects; 72 patients with ALS, 10 with benign fasciculations (these required normal MUP analysis) and 37 healthy control subjects (to establish our own normative values), matched for age. In the ALS group, 61 had normal strength

in the (TA), 9 patients had TA strength of MRC 4. In all subjects we evaluated the presence of FPs and fibs-sw, quantified MUPs (20 MUs) and evaluated MU stability (jitter). Sixteen ALS patients with clinically normal TA were investigated in serial studies.

Results: FPs were recorded in TA muscles (MRC 5) of 13 patients with ALS in whom there were no EMG features of neurogenic change. In four additional patients with FPs increased jitter was the only other abnormality. Of 20 TA muscles in which no FPs were detected at the initial assessment, 15 showed increased jitter, 11 showed fibs-sw and 16 had abnormal MUP parameters. Longitudinal studies confirmed that the patients presenting with FPs as the only abnormality progressed to MUP instability before large MUPs associated with fibs-sw were detected in the TA muscle. FPs from ALS patients with no other neurophysiological change were simpler than in patients in whom there were also fibs-sw and neurogenic MUPs ($p < 0.01$). The complexity of FPs in patients with weak TA was higher than in the latter group ($p < 0.01$). On the other hand, FPs from patients with benign fasciculations were simple (less complex) than those in ALS patients with normal TA strength ($p < 0.01$).

Discussion: Our results show that FPs are a very early marker of ALS and anticipate MUP instability. These findings are consistent with a very early distal axonopathy in ALS, but do not exclude an origin of FPs from a dysfunctional motor neuron cell body. Later, widespread neuronal death implies easy recognition of fibs-sw and reinnervation, at a phase of early compensatory reinnervation. Our results suggest that benign FPs represent a different phenomenon and confirm the importance of FP morphology analysis in the differential diagnosis of ALS and other disorders.

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C93 MOTOR UNIT NUMBER INDEX (MUNIX) LONGITUDINAL MEASUREMENTS IN ALS PATIENTS IN A MULTICENTRE TRIAL

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Keywords: MUNIX, ALSFRS-R, biomarker

Background: In previous studies motor unit number index (MUNIX) has shown to be a reliable and feasible motor unit number estimation (MUNE) method applicable in different muscles in ALS patients and has been suggested as a surrogate marker in clinical ALS trials. MUNIX is calculated by compound muscle action potentials (CMAP) and surface electromyographic recordings at different voluntary force levels. In comparison to other MUNE methods, MUNIX is non-invasive and quickly recorded.

Objective: To evaluate if MUNIX is a more sensitive marker of disease progression compared to ALSFRS-R score in a multicenter natural history study of ALS patients.

Methods: In three participating centers 31 ALS patients (18 limb, 13 bulbar onset) were recruited till May 2012. MUNIX measurements were performed every three months in six muscles of the clinically less affected side: biceps (BB), abductor digiti minimi (ADM), abductor pollicis brevis (APB), tibialis anterior (TA), abductor hallucis (AH) and extensor digitorum brevis (EDB). Additionally ALSFRS-R score was evaluated.

Results: After a 12 month period all measures declined relative to baseline between 21% (MUNIX AH) and 57% (MUNIX ADM). ALSFRS-R score declined 25%. Decline reached significance for ALSFRS-R after 12 months ($p < 0.001$). Decline of MUNIX was already significant after six months for the APB ($p < 0.006$), TA ($p < 0.009$) and EDB ($p < 0.03$) and after nine months for the ADM ($p < 0.00001$), AH ($p < 0.003$) and BB ($p < 0.04$). The most prominent difference of decline after 12 months between ALSFRS-R and MUNIX was seen for the ADM with 33% ($p < 0.005$) and the APB with 29% ($p < 0.02$) lower mean values. In bulbar onset patients a pronounced decline of MUNIX values was seen after 6 months.

Discussion and conclusions: The decline of MUNIX values over a 12-month period is significantly greater than decline of ALSFRS-R score, even when the clinically less affected side is examined. This indicates that motor neuron loss is already detectable with MUNIX before functional loss and reduction of ALSFRS-R score occurs. It also suggests that MUNIX is a sensitive electrophysiological outcome measure/biomarker which could be applicable in longitudinal multi-center ALS studies. One outstanding advantage of this method is the possibility to examine non-invasive multiple muscles in an adequate amount of time.

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C94 IS SERUM CREATININE A BIOMARKER FOR ALS?

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Keywords: biomarkers, clinical trials, trial design

Background: Developing biomarkers for ALS is a high priority for research. Serum creatinine is reduced in many patients with ALS, and it was previously found to correlate with survival in ALS(1).

Objectives: 1) To determine whether level of serum creatinine is associated with survival in ALS patients; and 2) to measure correlations over time between creatinine and ALSFRS-R as well as FVC.

Methods: We used Cox proportional hazards models applied to data from two clinical trials conducted by the WALS group to quantitate the relation between baseline creatinine and tracheotomy-free survival. We calculated baseline correlations for creatinine levels with ALSFRS-R, FVC, age and symptom duration. Also, for each patient we calculated the within-patient creatinine correlations over time with ALSFRS-R and FVC.

Results: At study entry levels of creatinine ranged from 0.3 to1.5 mg/dl for 315 patients with median follow-up of 13.7 months. A Cox model with creatinine as the only

predictor estimated a hazard ratio (HR) = 0.31 (p = 0.016). For 73 patients with creatinine < 0.7, median survival was 18 mo; for those with creatine ≥ 0.7, median survival was 26 mo (p = 0.0001 log-rank test). Baseline creatinine remained as a significant factor in a Cox model that also included patient age, symptom duration, initial ALSFRS-R and FVC (HR = 0.35, p = 0.024 for creatinine).

We found significant baseline correlations for creatinine with ALSFRS-R (r = 0.35, p < 0.01), FVC (r = 0.14, p = 0.01) and symptom duration (r = -0.17, p < 0.01) but not with age.

The median correlation *measured over time* for each patient between creatinine and ALSFRS-R was 0.39 (90% range −0.55 to + 0.94) while that for FVC was 0.38 (range −0.52 to + 0.93); both are significantly lower than the median 0.74 (range −0.09 to + 0.94) for ALSFRS-R and FVC.

Discussion: Our finding of shortened survival with lower baseline levels of circulating creatinine is consistent with the findings of Paillisse *et al.* (1) who reported it as a significant factor in a multivariate survival prognosis model. Our measurement of correlations between creatinine and other commonly used measures of ALS progression has not been previously studied. Recent studies showing the negative prognostic impact of weight loss also might bear on these results. These findings suggest that serum creatinine deserves further study as a potential biomarker, perhaps in combination with other putative biomarkers.

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C95 A NOVEL BIOMARKER STRATEGY FOR PREDICTING ALS PROGNOSIS

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Keywords: biomarker, prognosis, predictive model

Background: Both patients with ALS and their physicians would benefit from improved prognostic information. Currently, neither clinical nor laboratory measures permit accurate estimation of prognosis. Investigating biomarker profiles in ALS patients may yield better prognostic tools and elucidate disease pathways.

Objectives: To identify a panel of cytokines, trophic factors, and metabolites in blood, cerebrospinal fluid (CSF), and muscle whose levels are correlated with clinically meaningful prognostic measures in patients with ALS.

Methods: Samples of blood, CSF, and muscle were analyzed from ALS patients with definite, probable, probable laboratory-supported, or possible ALS as part of previous research protocols. Multiplex analysis of plasma was conducted using the Bio-Plex Human 27-plex panel of cytokines and growth factors, with additional ELISA-based immunoassay analysis. For muscle biopsy tissue, levels of soluble superoxide dismutase (SOD1) protein were determined using immunoassay. For CSF samples, multiplex analysis was conducted using the Bio-Plex Human 27-plex panel, with additional ELISA-based analysis. Clinical data included date of ALS symptom onset, date of death, disease duration from onset of symptoms to death, date of initiation of noninvasive ventilation (NIV), and date of gastrostomy tube placement. Pearson's correlations were performed using GraphPad Prism 4, while multiple regression was conducted using StatView 5. The use of the samples was approved by our Institutional Review Board.

Results: Six plasma biomarkers were significantly correlated with percent of diseased lifespan remaining, defined as the percentage of disease duration remaining at the time of sample collection (determined retrospectively). A multivariate model incorporating this biomarker panel predicted actual clinical values with R-squared = 0.794 (P < 0.0001, n = 23). Soluble SOD1 levels in muscle significantly correlated with percent of diseased lifespan remaining, and a univariate model predicted actual clinical values with R-squared = 0.530 (P = 0.0198, n = 12). The correlation was lost when individuals harboring H63D HFE polymorphism were included. A multivariate model developed to predict time from symptom onset to insertion of gastrostomy tube using six CSF biomarkers had predictive R-squared = 0.742 (P < 0.0001, n = 14). A multivariate model developed to predict time from symptom onset to initiation of NIV using two plasma biomarkers had predictive R-squared = 0.627 (P = 0.0063, n = 10). In general, the biomarkers identified were associated with pro-inflammatory processes.

Discussion and conclusions: Although the sample sizes in this study were small, these findings strongly support the potential usefulness of biomarker discovery efforts, and may improve understanding of ALS pathophysiology by identifying potential disease mechanisms. The biomarker panels reported here may provide prognostic value in ALS, which may benefit patient and caregiver planning, permit ALS health care teams to optimize treatment recommendations, and guide future research.

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C96 BODY FLUID BIOMARKERS FOR MOTOR NEURON DISEASE

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Keywords: biomarkers, urine, ELISA

Background: Biomarkers are urgently required for trials of potential therapies in humans with motor neuron disease (MND) and pre-clinical trials in animal models of MND such as SOD1G93A mice (1). The common neurotrophin receptor p75 (p75NTR) is highly expressed in motor neurons during embryonic development, but down regulated after birth. However, p75NTR is up-regulated in motor neurons after injury including MND patients and SOD1G93A mice (2, 3). We have previously found by Immunoprecipitation/Western-blot that extracellular p75NTR is significantly higher in urine of sporadic MND patients and symptomatic SOD1G93A mice than in healthy human and mouse controls (4). We now report the quantification and significance of urinary p75NTR in human MND and SOD1G93A mice.

Objectives: To determine if p75NTR levels in body fluids of MND patients and SOD1G93A mice could serve as a biomarker of MND.

Methods: Quantitative Sandwich ELISAs were developed to detect mouse and human p75NTR in the rg/ml range. Urine and serum was collected from patients with sporadic MND and from non MND diseases such as Parkinson's, Multiple Sclerosis, neuropathy and diabetes in addition to healthy controls (n = 10 for each group). Mass spectroscopy (Thermo Orbitrap) was used to confirm the presence of urinary p75NTR from an MND patient. Riluzole (210 mg/kg per week) trials in SOD1G93A mice are in progress, with urine and blood being collected to compare p75NTR levels in treated with non-treated mice.

Results: A novel sensitive sandwich ELISA for p75NTR was assessed for sensitivity and signal to noise (S/N) ratio. It was found that both mouse- and human-derived forms of p75NTR were detectable in the range 5–400 rg/ml, with high signal to noise ratios (S/N > 20) achieved. Humans diagnosed with early to mid stage sporadic MND had 2 to 5 fold more p75NTR than controls; n = 7. Three unique peptides of human p75NTR were identified from MND patient urine by mass spectroscopy. Analysis of urinary p75NTR from people living with other neurological conditions is continuing. p75NTR was detectable in healthy SOD1G93A mice (40 to 60 d), and increased until end-stage (145–160d; n = 6). Experiments are underway to analyze p75NTR in urine of SOD1G93A mice treated with riluzole.

Discussion and conclusion: Urinary p75NTR shows promise as a biomarker for human sporadic MND. Further work is ongoing to correlate p75NTR levels with disease progression so that it can be used to monitor effectiveness of therapies.

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THEME 1 THERAPEUTIC STRATEGIES

P1 HUMAN ADIPOSE-DERIVED STEM CELLS ENHANCE THE GLUTAMATE UPTAKE FUNCTION OF GLT1 IN SOD1(G93A)

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Keywords: adipose-derived stem cells, glutamate transport 1, SOD1(G93A)

Background: Impaired glutamate uptake function of astrocytes associated with the accumulation of extracellular glutamate is a well-documented feature of amyotrophic lateral sclerosis (ALS). Enhancing the uptake function of astrocytic glutamate transport 1 (GLT1) may be a potential treatment for this disease. Human adipose-derived stem cells (hADSCs) are capable of secreting a large number of cytokines which exhibit diverse pharmacological effects.

Objective: To investigate the influence of the soluble factors released by hADSCs on the GLT1 in primary astrocytes cultured from SOD1 (G93A) mice, a widely studied mutant human SOD1 transgenic model of ALS.

Method: 1) Animals:Transgenic mice of the strain B6SJL-TgN (SOD1-G93A) 1GUR (No. 002726), were purchased from the Jackson Laboratory. 2)Isolation and culture of hADSCs: Human subcutaneous adipose tissue samples were collected from patients undergoing liposuction surgery after obtaining informed consent. 3) Primary spinal cord astrocytes culture: Primary mouse spinal cord astrocytes were prepared from postnatal day (P) 0 to P2 SOD1G93A mouse pups or matched non-transgenic littermates. 4) Human ADSCs and astrocytes co culture Transwell chambers with a 0.4 lm pore size membrane were used to physically separate the astrocytes from hADSCs. 5) Immunophenotype analysis: Flow cytometry (FCM) was used to analyze the immunophenotype of hADSCs. 6) Confirmation of multilineage differentiation of hADSCs: Human ADSCs were analyzed for their capacity to differentiate toward the adipogenic and osteogenic lineages. 7) Immunocytochemistry. 8) Western blot analysis. 9) Glutamate uptake assay. 10) Caspase-3 activity assay. 11) Quantitative real-time PCR assay. 12) Statistical analysis.

Results: Our data indicate that soluble factors from hADSCs significantly upregulate the expression of GLT1 in SOD1(G93A)-bearing astrocytes, which result in enhanced glutamate uptake function. The upregulation of GLT1 is accompanied by the inhibition of caspase-3 activation in mutant astrocytes. In addition, we find that hADSCs co cultured with SOD1 (G93A)-bearing astrocytes produce more VEGF, HGF and IGF-1, which are reported to have neuroprotective effects.

Conclusion: Our results suggest that hADSCs may be a potential candidate in cellular therapy for ALS.

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P2 INTRATHECAL TRANSPLANTATION OF HUMAN STEM CELLS IN TRANSGENIC AMYOTROPHIC LATERAL SCLEROSIS MOUSE MODEL

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Keywords: human stem cells, human amniotic fluid cells, human umbilical cord cells

Stem cell (SC) transplantation is a potential strategy for neurological diseases and SC research has expanded for Amyotrophic Lateral Sclerosis (ALS). Intrathecal injection is less invasive and is capable of extensively delivering cells by cerebrospinal fluid.

Human amniotic fluid (hAF) or human umbilical cord (hUC) Wharton's jelly-derived SCs, and vehicle as controls, were delivered intrathecally at the lumbar intervertebral space of $SOD1^{G93A}$ ALS mouse model (transgenic, TG), and also of wild-type (WT) mice, approximately 15 days before disease onset. Mice were followed behaviorally and spinal cord tissue was examined (histopathology and western blot) either at the onset or at the endpoint, as performed in separate groups of animals.

Bisbenzimide-positive SCs were found in the lumbar pial meninge and adjacent spinal cord white matter of SC-injected mice. hAF SCs, but not hUC cells, delayed disease onset by about 5 days, and also led to higher neurological scores and better hanging wire and rotarod performances throughout analysis. ChAT positive motoneuron counting in the mouse lumbar spinal cord ventral horn showed that hAF SCs fully counteracted and hUC SCs partially counteracted the cell number diminution found in the control TG vehicle mice in relation to WT already at the onset of disease. However, such motoneuron protections disappeared at the endpoint. GFAP immunoreactive astroglial profiles increased in the anterior horn of the gray matter of all groups of ALS mice compared to WT at the onset and final stage of disease. In line with ChAT neuronal countings, the levels of ChAT protein were found to be maintained only in the hAF SCs group at the onset, as compared to WT, in contrast to the massive reductions found in the hAF SCs group at the final stage and also in the hUC SCs at the two studied periods. Interestingly, hAF SCs fully counteracted the ability of TG mice to trigger astroglial activation at disease onset, but not at the end point, an event not found after hUC SCs treatment both at the onset and at endpoint as evaluated by means of GFAP immunoblotting. Moreover, OX-42 western blot showed that hUC SCs potentiated the ability of TG mice to trigger microglial activation at the disease onset, but not at the endpoint. hUC SCs injection increased the protein levels of 21 and 23 kDa FGF-2 isoforms at the onset compared to WT group, however 23 kDa isoform was found to be elevated in the hUC SCs group compared to

hAF treatment. A single intratecal lumbar injection of hAF SCs may have promoted transient behavior and motor neuron protections in the ALS mouse model, events that may be related to the ability of hAF cells to counteract astroglial activation in early phases of disease. Support: FAPESP, CNPq, CAPES

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P3 ASSESSMENT OF HUMAN FETAL NEURAL STEM CELLS IN THE ALS TRANSGENIC MOUSE MODEL

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Keywords: SOD1 (G93A) mice, neural stem cells, survival study

Background: Administration of stem- or progenitor cells is currently being investigated as novel therapeutic option in ALS and other neurodegenerative diseases. Several studies have already shown neuroprotective potential of several types of adult stem cells which were mainly attributed to their release of trophic factors. Due to their differentiation potential, neural stem cells of fetal origin are of particular interest for the therapy of neurological disorders.

Objectives: We evaluated the effects of direct intraspinal administration of human fetal neural stem cells (NSC) in the G93A mouse model of ALS at presymptomatic disease stage.

Methods: Human neural progenitor cells were derived from CNS tissue of aborted human fetuses (gestational week 10–18) with mother's consent. All experiments were approved by the Ethics Committee of the University of Leipzig, Germany and in accordance with all state and federal guidelines. One week before surgery, NSC were thawed and expanded at standard conditions in a reduced atmosphere (3% O_2). On the day of surgery they were diluted in sodium chloride to a volume of 100,000 cells per 1µl. The animals received 1µl bilaterally into the lumbar region of the spinal cord. Control animals received 0.9% sodium chloride as vehicle. Groups were further divided into a survival group and a group of animals that were sacrified at day 110 for immunohistological analyses. Animals of the survival groups were monitored for survival, general condition, weight and motor function (rotarod and footprint analyses). Additional animals were injected with GFP-labeled NSC and sacrified at different time points to monitor survival, migration and differentiation of injected cells.

Results: Intraspinal surgery was well tolerated by all animals. Significant improvement of NSC-treated mice was detected in motor performance tests and general condition scores. Survival analysis is still in progress. One week after surgery, GFP-labeled NSC were found along the puncture channel in the spinal cord tissue. Analysis of further time-points (2, 3, 4 and 6 weeks) as well as further histological analyses (co-localization of GFP-positive cells with neural and glial markers, motor neuron counts, quantitative analysis of astrocytosis and microgliosis) is still ongoing.

Discussion and conclusions: Intraspinal stem cell injection was shown to be safe and well tolerated in ALS transgenic mice and has already been shown to be feasible in ALS patients. In our study, we found positive effects of lumbar NSC-injection on motor performance and general condition. Further analysis needs to determine how long transplanted cells can survive in the spinal cord and what exactly underlies their beneficial effects. Possibly, repeated injections at more than one injection site will be necessary to maximize the protective potential of adult stem cells in ALS.

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P4 TRANSDUCTION OF CORTICOSPINAL MOTOR NEURONS BY AAV2 FOLLOWING DIRECT INJECTION INTO THE MOTOR CORTEX

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Keywords: gene therapy, corticospinal motor neurons, AAV

Background: The use of adeno-associated virus (AAV) in gene therapy has multiple advantages due to its long-term expression in the central nervous system (CNS) and low immunoreactivity in humans. AAV-mediated gene therapy strategies have been considered for neurodegenerative diseases such as Canavan's disease, Alzheimer's disease and recently, motor neuron diseases including amyotrophic lateral sclerosis (ALS). AAV-IGF increased the lifespan of the well-characterized $hSOD1^{G93A}$ ALS mouse model. AAV serotypes come in many "flavors" given by capsid engineering to improve transduction efficiency and cellular specificity. Targeting only specific neuron population without affecting other neuron types within the cerebral cortex represents a major obstacle for translational neuroscience.

Objectives: In this study, we investigated the specific tropism of AAV serotypes for corticospinal motor neurons (CSMN). Seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbor the eGFP gene were tested after direct injection into the layer V of the motor cortex. CSMN transduction was confirmed by CTIP2 expression and by retrograde labeling with red fluorescent microspheres injected into the corticospinal tract (CST). In addition, using immunocytochemistry approaches we determine other cell types transduced by AAV serotypes including neurons (NeuN), callosal projection neurons (SATB2), astrocytes (GFAP), microglia (Iba-1), and oligodendrocytes (OLIG2).

Results: We find that different AAV serotypes have varied tropism for different neural cell populations in the motor cortex. For example, AAV 2-1 and AAV2-9 transduce a mixed cell population, including astrocytes. AAV2-5 exhibits tropis for callosal projection neurons, whereas AAV2-2, AAV2-6, and AAV2-8 show relatively high tropism for CSMN. However, none of the serotypes examined have selective tropism for CSMN.

Discussion: We have previously reported that CSMN are specifically transduced by AAV following microinjection into the corticospinal tract that lies within the dorsal funiculus of

the mouse spinal cord. However, anatomical differences between the mouse and human motor neuron circuitry may hinder translation of this method to a therapeutic context for gene therapy in humans. Therefore, identification of AAV serotypes that can transduce specific neuron populations upon direct cortical injection would be invaluable for potential gene therapy approaches, and for building effective treatment strategies for ALS patients.

Conclusions: Here we report that AAV2-2, AAV2-6, and AAV2-8 exhibit tropism for large pyramidal neurons in layer V of the motor cortex, including CSMN. Further enhancement of their tropism specifically for CSMN by engineering elements of the capsid proteins is an attractive and promising avenue of future research.

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P5 RESETTING INTRACELLULAR PH AS A POTENTIAL THERAPEUTIC APPROACH FOR AMYOTROPHIC LATERAL SCLEROSIS TESTED IN G93A MUTANT MICE

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Keywords: intracellular pH, motoneuron degeneration, mSOD1 mouse

Background: Studies on neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), have revealed several well-defined pathogenic processes such as excitotoxicity, reactive oxygen species (ROS) and apoptosis. It is also becoming clear that these pathogenic processes may lead to intracellular acidification that in turn accelerates pathological progress of the diseases. While the efforts in preventing and treating the neurodegenerative diseases have produced limited success, much less attention has been made to work on homeostasis of intracellular pH (pHi) in these diseases, and none has been done on ALS to our knowledge. On the other hand, intracellular alkalinization has been implicated in preventing cell death. We thus hypothesize an alkalic shift of pHi in motoneurons will benefit survival of ALS patient.

Objectives: The objective of this investigation is to evaluate therapeutic potential of resetting pHi using ALS mouse model (mutant G93A SOD1, mSOD1) by either acidification/alkalinization shifting of pHi, or in combination with riluzole. We focus on the effects of pHi on survival, behavior and spinal motor function ALS animal model.

Methods: Female adult mSOD1 mice were divided into four groups treated with NH_4Cl, $NaHCO_3$, and $NaHCO_3$ plus riluzole in various combinations starting at age of 85 postnatal days. The end stage of the mSOD1 mice were determined by failure on a rotarod for less than 5 seconds examined on daily basis. The progress of the disease was evaluated by monitoring hindlimb tremor, muscle weakness and body weight once a week. And spinal motor function was evaluated with *in vitro* root reflex technique.

Results: The main finding is that $NaHCO_3$ treatment significantly prolonged the end stages of the mSOD1 mice (the end stage for control mice: 136.0 ± 10 days, mean $\pm$ SD, $n = 39$; for $NaHCO_3$: 149.6 ± 9.6 days, mean $\pm$ SD, $n = 12$; $p = 0.008$), appearing as a right shift in Kaplan-Meier survival (KMS) plot. Although no significance was found for NH4Cl treatment (133.4 ± 4.1 days, mean $\pm$ SD, $n = 12$), the pattern of its KMS plot appeared different from control, suggesting a more complicated process in acidification treatment.

Discussion: PHi value is known to be affected by extracellular pH. Since motoneurons in ALS degenerate due to pathological changes mostly occurred inside their cell body, the prolonged survival of mSOD1 mice treated with $NaHCO_3$ is likely achieved through the change of pHi. In addition, NH4Cl was reported to cause pHi alkalinization in acute *in vitro* study while its chronic application at system level was found to acidify the body, which may explain the special pattern for its KMS plot.

Conclusion: The data suggest direct alkalinization of pHi improves the survival of mSOD1 and deserves further investigation as a new therapeutic strategy.

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P6 ACTH (ACTHAR GEL): PRECLINICAL STUDIES IN THE G93A-SOD1 MOUSE MODEL OF ALS

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Keywords: adrenal corticotrophic hormone, preclinical, drug

Background: There is no effective treatment or cure for amyotrophic lateral sclerosis (ALS), where survival after diagnosis is usually less than 5 years**.** One of the common features of both sporadic and familial disease is that neuroinflammatory processes are evident with disease onset or shortly thereafter. ACTH (adrenal corticotropic hormone) has anti-inflammatory, neurotropic and myotropic effects, but has not yet been tested in an ALS mouse model.

Objectives: 1: Can ACTH be used as a therapy for neurodegenerative diseases like ALS? 2: What dose and route of administration (intramuscular (IM) or subcutaneous (SC)) provides favorable effects on disease onset/progression?

Methods: We used G93A SOD1 transgenic mice expressing a high copy number of the human gene, which develop ALS-like symptoms and pathology. All experiments were done under an approved ACUC protocol. ACTHar gel was provided by Questcor Pharmaceuticals (Anaheim Hills, CA). The preparation contains the active hormone (ACTH1-39) in a gelatin matrix (16%) at 400 U/mL. Mice were given the drug at different doses (120 U, 60 U/kg) by IM or SC routes either on an every other day basis or on a weekly regiment. Each arm of the trial had 5–10 animals and a separate control group of 20 animals who were given gelatin. We began drug administration in presymptomatic 60-day old animals and continued until end-stage. The mice were monitored for clinical symptoms such as tremor and hind limb paralysis. Rotarod testing was also done on a weekly schedule.

Results: The administration of ACTHar gel was well tolerated. Even with a fairly large dose (120 U/kg) the mice exhibited no acute side effects. All SC dose regiments were found to delay onset of disease by 12 to 19 days (log rank $P = 0.0001$. Onset of paralysis was also delayed by 6–8 days (log rank $p = 0.05$). However, survival was not extended significantly, (log rank $p = 0.313$) for any arm of the study.

Rotarod performance for all SC-treated animals improved significantly within a 4–5 week window (Weeks 9–14), $p < 0.01$ for males and 0.001 for female mice. Finally, the levels of G93A-SOD1 in the treated animal spinal cords were reduced by at least 75% ($p = 0.001$–0.01).

Discussion: ACTHar is FDA approved for treating relapsing multiple sclerosis, infant spasms, and nephritic syndromes. The tolerance to the drug and the significant effects on disease onset and early progression suggests that ACTHar gel has potential as a therapy for ALS. The surprising decrease in levels of SOD1 may add an additional benefit for patients with familial SOD1-linked disease.

Conclusions: Additional studies are in progress to optimize the dose of ACTHar gel to enhance survival as well as delayed onset and early progression.

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P7 DELETION OF THE BH3-ONLY PROTEIN BID INHIBITS ASTROCYTE AND MICROGLIA ACTIVATION AND DELAYS DISEASE PROGRESSION IN ALS MICE

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Keywords: astroglia, inflammation, BID

Background: ALS pathology is accompanied by glial cell activation and neuroinflammation. Increased levels of pro-inflammatory cytokines were proposed to mediate the neuroinflammation and resultant activation of apoptotic pathways via consecutive activation of death receptors, caspase-8 and the pro-apoptotic BH3-only protein BID.

Objectives: The aim of the current study was to examine the contribution of the BH3-only protein BID to motor neuron loss, ALS disease progression and pro-inflammatory signalling *in vitro* and *in vivo*.

Methods: RT-qPCR and Western blotting experiments were carried out on lysates from lumbar spinal cords to assess BID mRNA and protein levels across ALS disease progression in *SOD1*^{G93A} mice. BID deficient primary mixed motoneuron cultures were treated with pro-inflammatory cytokines to examine cytokine-induced signal transduction and cell death. KB-dependent reporter gene activation was monitored in transiently transfected mixed motorneuron cultures. We generated *bid*-deficient mice expressing human mutant *SOD1*^{G93A} and assessed for motor function, lifespan, and motoneuron survival ($n = 24$/group; age, gender (12 males/12 females), weight and litter-matched) in accordance with the most recent ALS guidelines for generating preclinical data (1)().

Results: *bid* mRNA levels were significantly increased across disease progression particularly at end stage and BID protein levels were significantly increased at PND 120. Interestingly, *bid* gene deletion protected primary mixed motoneuron cultures against interleukin-1β (IL-1 β) and interferon-γ (IFN- γ) mediated cell death. *In vivo*, genetic deletion of *bid* in *SOD1*^{G93A} transgenic mice delayed the onset and progression of the ALS phenotype. In addition, *bid* deficiency significantly increased motoneuron survival in *bid*$^{+/-}$ mice at end stage, and potently inhibited microglia and astrocyte activation. Reporter gene assays performed in mixed motoneuron cultures suggested that *bid* was indeed required for the efficient activation of the pro-inflammatory transcription factor nuclear factor-KB in response to pro-inflammatory cytokines, independent on its effect on cell death.

Discussions and conclusions: Our data suggest a dual role for BID as cell-death inducer and as an important mediator of inflammation and astrocytosis during ALS disease progression. These results are particularly interesting in view of recent findings (2) that suggested non-apoptotic roles of Bid in inflammation and innate immunity in colonocytes. In summary, our findings highlight BID as a possible therapeutic target in ALS.

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P8 THE NEUROPROTECTIVE COPPER-BIS(THIOSEMICARBAZONATO) COMPLEX, CUII(ATSM), INDUCES NRF2 ACTIVATION AND UPREGULATION OF ANTIOXIDANTS IN CULTURED ASTROCYTES: POTENTIAL MECHANISM OF ACTION IN TRANSGENIC MOUSE MODELS OF ALS

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Keywords: therapeutic, Nrf2, astrocyte

Background: We have shown that Cu^{II}(atsm) has therapeutic potential for the treatment of ALS. Cu^{II}(atsm) extends the lifespan, improves motor function and survival of motor neurons, decreases oxidative and nitrosative damage, and attenuates astrocyte and microglial activation in the SOD1G93A transgenic mouse model of ALS (1). Similar protective action has also been found in a SOD1G37R mouse model.

Objectives: This study seeks to elucidate the mechanism by which Cu^{II}(atsm) elicits these effects. As oxidative damage and astrocyte activation were attenuated by Cu^{II}(atsm), the potential stimulation of neuroprotective antioxidant systems of astrocytes was investigated in vitro.

Methods: Primary astrocytes cultured from mouse brains were treated with Cu^{II}(atsm) for up to 24 h. Biological activity of the compound was assessed by determining activation of signaling kinases by Western blot. Nrf2 is a predominantly glial transcription factor responsible for regulating antioxidant enzymes such as heme oxygenase-1 (HO-1) and

glutamate-cysteine ligase (GCL), the latter of which controls the synthesis of the critical antioxidant glutathione. Activation of Nrf2 was assessed by transfecting astrocytes with an antioxidant response element (ARE)-GFP reporter. Induction of the downstream Nrf2 targets HO1 and GCL were assessed by Western blot and activity assay, respectively. Glutathione content and export were also determined following Cu^{II}(atsm) treatment.

Results: Cu^{II}(atsm) caused phosphorylation of the signaling kinases Akt, ERK and JNK, indicating its biological activity. ARE-GFP fluorescence was increased with Cu^{II}(atsm) treatment, demonstrating activation of Nrf2. Cu^{II}(atsm) treatment also stimulated the downstream Nrf2 targets HO1 and GCL. Accordingly, glutathione content and export from astrocytes was also elevated by Cu^{II}(atsm) treatment. The latter is important, as glutathione export from astrocytes is essential to maintain the glutathione content of neurons.

Discussion and Conclusion: We have found that Cu^{II}(atsm) activates the transcription factor Nrf2 and upregulates the antioxidant systems of cultured astrocytes. This activation may contribute to the neuroprotective and disease-attenuating effects of Cu^{II}(atsm) observed *in vivo* in ALS model mice, and indicates that Nrf2 may be an important therapeutic target for the treatment of ALS.

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P9 BROMOCRIPTINE RETARDS DISEASE PROGRESSION IN AN ALS MOUSE MODEL VIA SUPPRESSION OF GLIAL INFLAMMATION

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Keywords: oxidative stress, astrocytes, mutant SOD1 transgenic mice

Background: Recent studies have suggested that oxidative stress plays a critical role in the progression of motoneuron loss in amyotrophic lateral sclerosis (ALS). Thus, oxidative stress could be a main target for the development of novel therapeutic agents in ALS. Neuronal apoptosis inhibitory protein (NAIP) has been shown to suppress cell death induced by oxidative stress and to exert neuroprotective activity. Based on the NAIP function, we originally developed the NAIP-ELISA-based drug screening system and screened 953 neurotropic compounds containing medical drugs by using this system. Among hit compounds, a dopamine D2 receptor agonist bromocriptine (BRC), which selectively exerts cell protection against oxidative insults and has been used as a treatment for Parkinson's disease, was of interest.

Objectives: The aim of this study was to evaluate the efficacy of BRC in a congenic ALS-$SOD1^{H46R}$ mouse model.

Methods: Intraperitoneal daily administration of BRC (0mg, 1mg and 10mg/5mL/kg) was commenced after the onset of symptom in ALS-$SOD1^{H46R}$ mice (post-onset administration). To validate the BRC efficacy in ALS-$SOD1^{H46R}$ mice, we performed behavioral (balance beam, vertical pole, and footprint tests) and neuropathological analyses. To evaluate the additive effect of riluzole, mice were treated with vehicle or BRC in combination with riluzole, and motor performance was assessed by balance beam and footprint tests.

Results: Behavioral analyses revealed that the post-onset administration of BRC sustained motor function in ALS-$SOD1^{H46R}$ mice at a symptomatic stage (21–22 weeks of age) when compared with the age-matched vehicle-treated ALS-$SOD1^{H46R}$ mice. The BRC treatment prolonged 12% of post-onset survival interval in ALS-$SOD1^{H46R}$ mice compared with vehicle control (p, 0.05 by log-rank test). However, there was no synergistic or additive effect of riluzole on motor function and disease progression in ALS-$SOD1^{H46R}$ mice. We demonstrated that the BRC treatment delayed ChAT-positive neuron loss and reduced the levels of activated astrocytes and inflammatory factors, iNOS and TNF-α, in the anterior horn of spinal cord in ALS-$SOD1^{H46R}$ mice. *In vitro* studies showed that the BRC treatment reduced the level of extracellular TNF-α in lipopolysaccharide-exposured mouse astorcytes. The ALS-$SOD1^{H46R}$ mice treated with BRC also exhibited the reduced level of oxidative damage. Further, the BRC treatment upregulated several anti-oxidative stress factors such as HO-1 and ATF3 in SH-SY5Y cultured neuronal cells via a dopamine receptor-independent pathway.

Discussion and conclusions: Our present study demonstrated that the post-onset treatment of BRC sustained motor functions, suppressed glial inflammation, and retarded the disease progression after onset in the ALS-$SOD1^{H46R}$ mouse model, implying that BRC protects motoneurons from the oxidative insults via suppression of astrogliosis in ALS-$SOD1^{H46R}$ mice. Thus, BRC is a highly promising drug for ALS.

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P10 SLOWING DISEASE PROGRESSION IN THE SOD1 MOUSE MODEL OF ALS BY BLOCKING NEUREGULIN

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Keywords: therapeutic target, neuregulin1, disease progression

Background: Neuregulin1 (NRG1) is both a membrane bound and secreted growth and differentiation factor that regulates glial development as well as survival, synaptogenesis, axoglial interactions, and microglial activation. We first reported aberrant NRG1 signaling in amyotrophic lateral sclerosis (ALS) (1). We have developed a targeted neuregulin antagonist called HBD-S-H4 (2) that given intrathecally reduces microglia activation in rat chronic spinal cord pain model (3). Therefore, here we hypothesize that blocking NRG1 with HBD-S-H4 could be a new potential therapeutic to slow disease progression in patients with ALS.

Objectives: We determined whether blocking NRG1 in the central nervous system slows disease progression and prolongs survival in the ALS-superoxide dismutase 1 (SOD1) mouse model.

Methods: The NRG1 antagonist HBD-S-H4 was delivered weekly through an implanted intracerebroventricular cannula for 9 weeks (started from 8 weeks of age at the preclinical stage to 16 weeks of age when disease onset showed in control ALS-SOD1 mice). Body weight, disease onset and progression, animal survival as well as pathological changes were measured in the HBD-S-H4 treated mice compared with saline-treated control group.

Results: We have recently shown that soluble forms of NRG1 are induced in human ALS and the SOD1 model and that NRG1 receptor activation on activated microglia is associated with disease progression. Our initial results show that weekly treatments for 9 weeks of recombinant HBD-S-H4 are not toxic, delayed disease onset, and prolonged survival in the SOD1 mice. Cellular pathological changes in HBD-S-H4-treated mice are underway.

Discussion and conclusions: We identified a common therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and ALS-SOD1 model. We are currently testing whether a NRG1 antagonist blocks microglial activation in the SOD1 model, as it does in other models and, whether this would be a potential therapeutic for patients with ALS.

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P11 THERAPEUTIC EFFECT OF GENETICALLY MODIFIED MUSCLE PROGENITOR CELLS IN ALS MICE

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Keywords: muscle progenitor cells, genetically modified cells, neurotrophic factors

Background: Neurotrophic factors (NTFs) preserved and protected motor neuron in ALS models. However, all the clinical studies with administration of NTFs in ALS patients failed. We have developed muscle progenitor cells (MPCs) populations expressing BDNF, GDNF, VEGF or IGF-1, (MPC-NTFs). Combined conditioned media collected from the cells rescued motor neuron cell lines (NSC-34) from various insults. Furthermore, MPC-NTFs 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, 118 of life. We found a significant delay of 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 MPCs over expressing just GDNF did not elicit any improvement. The results suggest a synergistic effect of the transplantation of MPCs expression in 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 increase the phosphorilated AKT by 6–8 folds compared to MPC expressing a single NTF.

Discussion and conclusions: Here we have built a novel powerful strategy that enables a stable, long-term administration of four NTFs cocktails. 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 release 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 inhibits 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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P12 TREATMENT WITH PYM50028 IMPROVES NEUROMUSCULAR FUNCTION IN A MOUSE MODEL OF ALS

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Keywords: muscle function, neuroprotection, motor units

Background: Mice expressing the mutant human SOD^{G93A} (mSOD1) have been extensively used to test drug candidates for ALS. In this study we examined the effects of a non-peptide neurotrophic factor inducer, PYM50028 (Phytopharm plc) on disease progression in mSOD1 mice. PYM50028 has been shown to be neuroprotective in a model of Parkinson's disease, where it induces the expression of BDNF and GDNF (1). Extracellular GDNF can prevent motoneuron degeneration (2). Therefore, trophic factor inducers that can increase endogenous GDNF production may be potential therapeutic candidates for ALS.

Objectives: To establish the effects of PYM50028 on neuromuscular function and motoneuron survival in mSOD1 mice.

Methods: The effects of PYM50028 in mSOD1 mice were compared to vehicle-treated mice, riluzole-treated mice, and mice treated with both PYM50028 and riluzole. There were 4 treatment groups (n = 15/group): 1) PYM50028; 2) Riluzole; 3) PYM50028 plus riluzole and 4) Vehicle. At 120 days of age mice underwent physiological assessment of hindlimb muscle force and motor unit survival. Motoneuron survival was assessed from fixed spinal cord sections whereas hindlimb

muscles (TA, EDL) were processed for histochemical analysis of succinate dehydrogenase (SDH) activity.

Results: There was a 40% improvement in hindlimb muscle force in 120 day old mSOD1 mice treated with PYM50028 compared to vehicle-treated mice. A similar improvement in muscle force was observed in mice treated with both PYM50028 and riluzole, although treatment with riluzole alone did not improve muscle force. In mice treated with PYM50028 alone or in combination with riluzole, there was a 10% and 15% increase in EDL motor unit survival, respectively. Treatment with riluzole alone failed to preserve any motor units. Furthermore, there was a significant increase in motoneuron survival in mice treated with PYM50028 alone or in combination with riluzole, so that, respectively, 40% and 50% more motoneurons survived than in vehicle-treated mice. Although riluzole had no detectable effects on muscle function, it did have a significant neuroprotective effect, and 30% more motoneurons survived in the riluzole only group than in the vehicle-treated group. SDH staining revealed extensive metabolic changes in 120 day old mSOD1 muscles which were not prevented by riluzole, but were almost completely prevented in the PYM50028-treated group.

Discussion: Our results show that PYM50028 improves muscle function and rescues motoneurons in mSOD1 mice. These effects are significantly greater than those of riluzole. The effects of PYM50028 in mSOD1 mice are not altered by co-treatment with riluzole. It is possible that the beneficial effects of PYM50028 in mSOD1 mice are due to its ability to induce the expression of neurotrophic factors.

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P13 DEVELOPMENT OF A C9ORF72 ALS ANTISENSE THERAPY AND A THERAPEUTIC BIOMARKER

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Keywords: C9ORF72, antisense, iPS

Background: A hexanucleotide 'GGGGCC' repeat expansion in the noncoding region of the *C9ORF72* gene has recently been identified in ~30% of familial and ~4–10% of sporadic ALS cases 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, FXTAS) and pathogenicity is thought to arise by aberrant binding of *trans*-acting factors to the *cis* repetitive elements. This is perhaps best studied in DM1 where MBNL1 is sequestered by the 'CTG' expansion in the *DMPK* pre-mRNA resulting in altered gene expression and aberrant splicing.

Objectives: To characterize expression and splicing patterns due to the presence of a 'GGGGCC' repeat in the non-coding region of the C9ORF72 gene and utilize these data to develop a viable antisense oligonucleotide (ASO) therapy and therapeutic readout.

Methods: Generate using high-throughput screening, profile, C9ORF72 patient fibroblasts, iPS cells, iPS-differentiated motor neurons and astrocytes, and human autopsy tissue. Utilize the developed C9ORF72 cell lines and test multiple ASO sequences for C9ORF72 knockdown and normalization of the identified dysregulated/mis-spliced transcripts.

Results: We have identified expression patterns and splice variants unique to cells/tissue that contain the C9ORF72 repeat expansion. Moreover, we have been able to utilize C9ORF72 siRNA/ASO knockdown methodologies to normalize specific CNS genes dysregulated in the C9ORF72 transcriptome.

Discussion and conclusions: We have generated and profiled a number of ALS patient-derived fibroblasts, iPS cells, and iPS-differentiated astrocytes/neurons and have identified unique expression and splicing patterns in cells that carry the C9ORF72 repeat expansion. We have further validated these data using autopsy tissue from human C9ORF72 ALS patients. Since blocking the repeated *cis* elements by ASO and RNA knockdown application is thought to be viable therapeutic in other neuromuscular repeat expansions disorders (eg, DM1), we tested this possibility in patient-derived C9ORF72 cells. Using these techniques, we are able to knockdown C9ORF72 RNA and protein levels. Furthermore, our transcriptome profiling yielded a number of CNS-expressed gene targets, whose aberrant expression in C9ORF72 cells can be rescued with ASO/siRNA treatment suggestive of ideal biomarker candidates. These approaches will allow us to effectively develop 1) antisense-mediated therapeutic approaches to ALS and 2) a relevant pharmacodynamic readout for antisense efficacy.

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P14 TARGETING THE ENDOGENOUS HEAT SHOCK RESPONSE AS A THERAPEUTIC APPROACH IN SPINAL BULBAR MUSCULAR ATROPHY (SBMA)

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Keywords: animal model, Kennedy's Disease, motoneuron survival

Background: Spinal and bulbar muscular atrophy (SBMA), otherwise known as Kennedy's disease, is an X-linked, late-onset progressive neurodegenerative disease, which predominantly affects males. Pathologically, the disease is defined by selective loss of spinal and bulbar motoneurons with accompanying neuromuscular impairment. Molecularly, the disease results from an expansion in the CAG repeat in the androgen receptor (AR) gene which encodes a polyglutamine tract in the mature protein (1, 2). The polymorphic CAG repeat normally ranges from 9 to 36, but an expansion of greater than 38 repeats results in disease. Although the underlying pathophysiology of the disease remains largely unknown, it is related to abnormal nuclear

accumulation of the pathogenic AR protein. Several treatment strategies for SBMA have therefore focused on decreasing nuclear accumulation and protein misfolding. We have previously shown that treatment with arimoclomol, a pharmacological co-inducer of the heat shock response, reduces the formation of ubiquitinated inclusions, alleviates disease symptoms and increases lifespan in the SOD1^{G93A} mouse model of ALS. In this study, we examined the effects of treatment with arimoclomol in a mouse model of SBMA in which mice carry 100 CAG repeats (AR100) in the human AR gene (3). These mice recapitulate typical hallmarks of the human disease, including motoneuron loss and accompanying neuromuscular deficits.

Objectives: To test the effects of arimoclomol on disease progression in SBMA mice.

Methods: Arimoclomol (120mg/kg/day) was administered orally (in drinking water) to male mice, after symptom onset, from 12 months of age. At 18 months, mice were anaesthetised with isoflurane and the distal tendons of the tibialis anterior (TA), extensor digitorum longus (EDL) and soleus hindlimb muscles cut and attached to force transducers. The sciatic nerve was exposed and stimulated to elicit muscle contraction. Muscle force, contractile characteristics and motor unit survival was determined in untreated and arimoclomol-treated AR100 and wildtype (WT) mice. Furthermore, the survival of spinal motoneurons was determined morphologically.

Results: Arimoclomol significantly improved hindlimb muscle force and contractile characteristics, rescued motor units and importantly, improved motoneuron survival in SBMA mice treated from 12–18 months of age.

Discussion: Upregulation of the heat shock response by treatment with arimoclomol after symptom onset may have therapeutic potential in the treatment of SBMA.

Conclusions: Since SBMA is a hereditary disorder, presymptomatic individuals carrying the mutation can be genetically identified. We are therefore currently investigating the effects of presymptomatic treatment of SBMA mice with arimoclomol (from 6 months of age), to establish whether this regime is more effective than that observed following treatment after symptom onset.

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P15 SYSTEMIC ANGIOGENIN DELIVERY AS A THERAPY FOR PATIENTS WITH ALS

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Keywords: angiogenin, therapeutics, SOD1 mouse

Background: A previous study has identified mutations in a gene encoding for angiogenin in ALS patients (1). Subsequent studies by our group demonstrated that angiogenin protects cultured motoneurons against ALS-associated, stress-induced cell death (2,3). Furthermore, we have demonstrated that systemic angiogenin protein delivery significantly increased life-span and improved motor function in SOD1G93A mice (3). These results also suggested that angiogenin protein delivery may be beneficial in treating patients with newly diagnosed ALS.

Objectives: The aim of the current study was to focus on developing these findings into a therapeutic technology based on the delivery of systemic angiogenin protein for the treatment of ALS and to investigate if angiogenesis is related to angiogenin's neuroprotection in ALS.

Methods: We examined the effect of systemic delivery of angiogenin on angiogenin serum levels and uptake in a mouse model of ALS, the SOD^{G93A} mouse. We also assessed whether angiogenin treatment increases angiogenesis and vascularisation in SOD^{G93A} mouse. We expanded the study to include a comprehensive dose-response investigation of the effect of systemic angiogenin protein delivery on life-span and disease progression and motor function in a post-symptom onset treatment paradigm in both SOD^{G93A} and TDP-43 mice (n = 24/group; age, gender (12 males/12 females), weight and litter-matched) in accordance with the most recent ALS guidelines for generating preclinical data (4).

Results: Our pharmacokinetic studies demonstrated a marked difference in angiogenin uptake and elimination in the SOD1G93A mice compared to their wild-type counterparts. Additionally, angiogenin uptake and angiogenesis was observed in the spinal cord of SOD1G93A mice following systemic administration of angiogenin. Dose-response studies demonstrate an extension in lifespan, an increase in motor function and motoneuron survival in mice models of ALS following systemic angiogenin treatment from our post-symptom onset treatment paradigm.

Conclusion: Together, our data suggest a role for angiogenesis the role of angiogenin and generate a comprehensive preclinical package for developing angiogenin as a therapy for clinical evaluation in ALS.

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P16 A MULTICENTER SCREENING TRIAL OF THE SAFETY AND EFFICACY OF RASAGILINE IN PEOPLE WITH ALS

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Keywords: rasagiline, oxidative stress, mitochondrial dysfunction

Background: Despite multiple clinical trials and recent advances in understanding its pathogenesis, there is no cure or effective treatment for amyotrophic lateral sclerosis (ALS). Oxidative stress, mitochondrial dysfunction and apoptosis have been proposed as the cause of motor neuron death in ALS.

Rasagiline, a monoamine oxidase B inhibitor, is FDA-approved for the symptomatic treatment of Parkinson's disease. Rasagiline has demonstrated broad neuroprotective activities against a variety of neurotoxins in neuronal cell cultures and in the SOD mouse model of ALS. *in vitro* experiments indicate rasagiline stabilizes mitochondria under stress conditions.

Objectives: Specific Aim 1: To determine whether rasagiline is safe in this patient population and if the drug has the potential to slow ALS disease progression. Specific Aim 2: To determine if mitochondrial function is affected by rasagiline. We will measure and compare the change of BCL-2/BAX prior to, before and after the rasagiline treatment study drug.

Method: This is a phase II multi-center open label study in El Escorial probable or definite ALS who met our inclusion and exclusion criteria. Subjects are treated with rasagiline 2 mg daily for 12 months. The primary outcome measure is the change of the slope of ALS Functional Rating Scale-Revised (ALSFRS-R) over 12 months as compared with natural history data derived from four large completed ALS trials. The secondary outcome measure is the change in proposed biomarkers including blood leukocyte Bcl-2/Bax ratio, mitochondrial potentials in platelets, and indicators of apoptosis and oxidative stress following rasagiline administration. We are also evaluating safety laboratory and clinical data.

Results: Nine centers in the Western ALS (WALS) study group are participating in this study. These centers recently completed enrollment of thirty-five ALS patients. There were 18 men and 17 women, with a mean age at entry of 61 years (59–82), and a mean disease duration of 1.5 years (0.5–3). The mean ALSFRS-R at study entry was 38.6 (31–45). Currently, rasagiline 2 mg is safe and well tolerated in patients with ALS. However, four patients did drop out due to side effects (dizziness) of the medication and there was one patient death unrelated to the study drug.

Conclusion: Six month data on primary and secondary outcome measures as well as safety data will be available before the meeting and will be presented.

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P17 DEXPRAMIPEXOLE IS NOT CONVERTED TO PRAMIPEXOLE AFTER ADMINISTRATION IN HUMANS

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Keywords: dexpramipexole, pramipexole, chiral interconversion

Background: Dexpramipexole (DEX) is currently being investigated in clinical studies for the treatment of amyotrophic lateral sclerosis (ALS). DEX is the R-(+) enantiomer of pramipexole (PPX), which is a non-ergoline dopamine agonist marketed (Mirapex®) for the treatment of Parkinson's disease and restless legs syndrome. Although DEX and PPX may share neuroprotective properties, DEX is pharmacologically distinct from PPX in that DEX has minimal affinity for dopaminergic receptors.

Objective: This study was designed to determine whether PPX can be identified in the circulation at pharmacologically significant levels following oral administration of DEX in humans.

Methods: Healthy adult subjects were administered doses of DEX up to 600 mg. A plasma sample was taken 2 hours after dosing. To monitor potential circulating PPX, a highly sensitive and selective chiral LC-MS/MS assay was developed and qualified for the detection of PPX in the presence of DEX in human plasma. In this assay, human plasma samples were spiked with an isotope-labeled internal standard solution. Plasma samples were extracted by solid phase extraction (SPE). The analyte PPX was separated from DEX using a chiral HPLC method. An LC-MS/MS system consisting of a Shimadzu LC20-ADXR Prominence UFLC and AB Sciex triple quadrupole mass spectrometer was used. Multiple-reaction monitoring (MRM) and electrospray positive ionization were used for analyte detection. For the qualified assay, the dynamic range of PPX was 0.150–1.00 ng/mL with the lower limit of quantitation at 0.150 ng/mL in the presence of up to 1000 ng/mL of DEX. The minimal pharmacologically active PPX concentration is expected to be greater than 0.300 ng/mL (1). Intra- and inter-day precisions and accuracies were within 80–120% of nominal values for both standards and quality control samples.

Results: A total of 16 samples were tested to determine the presence of pharmacologically significant PPX. PPX was not detected in any of the samples tested (<0.300 ng/mL in the presence of up to 1860 ng/mL of DEX).

Discussion and conclusions: PPX was not detected in humans at pharmacologically significant levels in human plasma after administration of dexpramipexole at doses up to 600 mg/day. Therefore, chiral conversion of DEX to PPX, if it occurs at all, occurs very rarely and does not lead to pharmacologically active PPX in vivo.

Reference

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P18 A MULTICENTER, OPEN-LABEL, SINGLE-DOSE, PHARMACOKINETIC AND SAFETY STUDY OF DEXPRAMIPEXOLE IN HEALTHY SUBJECTS AND SUBJECTS WITH RENAL IMPAIRMENT

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Keywords: dexpramipexole, pharmacokinetics, renal impairment

Background: Dexpramipexole is currently being investigated for the treatment of amyotrophic lateral sclerosis (ALS). Dexpramipexole has been shown to be primarily renally eliminated and exhibits linear pharmacokinetics across a wide dose range (1).

Objectives: The main objective of this study was to evaluate the pharmacokinetics of a single oral dose of dexpramipexole in healthy subjects and in those with varying degrees of renal impairment. Secondary objectives included a) establishment of a relationship between estimated glomerular filtration rate (eGFR) and dexpramipexole pharmacokinetics and b) evaluation of the safety and tolerability of dexpramipexole in subjects over a range of renal function.

Methods: This was a Phase I, multicenter, open-label, single-dose study. Adult subjects were recruited following an initial screening visit at which a screening eGFR was calculated based on the Modification of Diet in Renal Disease (MDRD) equation. Subjects were grouped by renal function (mild (eGFR 50–79 mL/min/1.73m^2), moderate (eGFR 30–49 mL/min/1.73m^2), severe (eGFR $<$ 30 mL/min/1.73m^2), end-stage renal disease (ESRD, on hemodialysis for at least 3 months)). Twelve healthy volunteers (eGFR $\geq$ 80mL/min/1.73m^2) were matched by age ($\pm$ 10 years) and gender to subjects with renal dysfunction. Each subject received a single dose of dexpramipexole on Day 1; subjects with mild (n = 6) or moderate (n = 6) renal impairment and matched healthy volunteers (n = 8) received 150 mg and subjects with severe renal impairment (n = 6) or with ESRD (n = 6) and matched healthy volunteers (n = 4) received 75 mg (administered day after dialysis for subjects on hemodialysis). In order to identify any unexpected safety or tolerability issues, subjects (referred to as Sentinel) were selected for each renal function cohort (n = 2 for mild and moderate renal impairment and n = 1 for severe and ESRD). Sentinel subjects then progressed through the entire study and had results analyzed prior to dosing the remaining subjects. Urine and blood samples were collected before dosing and for 72 hours post-dosing to determine dexpramipexole concentration. Additional blood samples were collected up to 144 hours in patients with severe renal impairment and ESRD. Laboratory tests, electrocardiograms, vital signs, and adverse event reports were collected to determine safety. Assessments were done for all patients before dosing and on Days 1–4 and Day 7. Patients with ESRD were also assessed on Days 5 and 6.

Results: 36 subjects completed the study as planned. The data are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters and safety and tolerability of dexpramipexole in healthy subjects and in subjects with mild, moderate, severe, or ESRD will be reported.

Reference

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P19 A RANDOMIZED, BLINDED, PLACEBO-CONTROLLED ASCENDING DOSE STUDY OF THE SAFETY AND PHARMACOKINETICS OF DEXPRAMIPEXOLE IN HEALTHY VOLUNTEERS

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Keywords: dexpramipexole, pharmacokinetics, safety

Background: Dexpramipexole, a synthetic amino-tetrahydrobenzothiazole, is being evaluated for the treatment of amyotrophic lateral sclerosis (ALS).

Objectives: The primary objective of this study was to determine the safety and tolerability of oral dexpramipexole in doses up to 600 mg as a single dose or 300 mg twice daily for 3.5 days in healthy volunteers. The secondary objective was to evaluate the pharmacokinetics of these doses.

Methods: This Phase 1, single-center, blinded, randomized, placebo-controlled, ascending-dose study was conducted in two parts using 5 cohorts (each cohort was composed of nine healthy adults including at least four females). Randomization within each cohort was 7: 2 dexpramipexole to placebo. Part A (3 cohorts) was a single-ascending dose study (SAD) and Part B (2 cohorts) was a multiple-ascending dose study (MAD). A screening visit to determine study eligibility was conducted in the 28 days prior to dosing. In Part A, each subject received a single oral dose of dexpramipexole (300, 450, or 600 mg) or placebo on Day 1. Serial blood and urine samples were collected prior to dosing and for 72 hours after dosing to determine dexpramipexole concentration. Safety was assessed for all subjects (adverse events, vital signs, clinical laboratory evaluations, electrocardiograms, physical examinations) before dosing, on Days 1–4 and on Day 8 ($\pm$ 1 day). In Part B, subjects received twice daily doses of dexpramipexole (225 or 300 mg) or placebo on Days 1 to 3 and a single dose on the morning of Day 4. Serial blood and urine sampling was done prior to dosing, for up to 12 hours following the initial dose, and for 72 hours following the last dose. Safety assessments were conducted for all subjects before dosing, on Days 1–7, and on Day 11 ($\pm$ 1 day).

Results: A total of 45 subjects completed the study. The data are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters following single and multiple doses and safety of up to 600 mg dexpramipexole in healthy volunteers will be reported.

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P20 A SINGLE AND MULTIPLE DOSE, OPEN-LABEL STUDY OF THE PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF DEXPRAMIPEXOLE IN HEALTHY JAPANESE AND CAUCASIAN SUBJECTS

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Keywords: dexpramipexole, pharmacokinetics, Japanese

Background: Dexpramipexole is a potential first-in-class mitochondrial modulator being developed for the treatment of amyotrophic lateral sclerosis (ALS). An ongoing Phase 3 study (EMPOWER) is evaluating the use of dexpramipexole 150 mg twice daily for ALS. Dexpramipexole pharmacokinetics in the Japanese population has not been investigated.

Objectives: The primary objective of this study was to evaluate the pharmacokinetics of single and multiple doses of dexpramipexole in healthy Japanese and Caucasian subjects. A secondary objective was evaluation of the safety and tolerability of single and multiple doses of dexpramipexole in these subjects.

Methods: This study was conducted in 2 parts; for each part, a screening visit was done within 28 days prior to dosing. Subjects of Japanese and Caucasian descent were enrolled in a 1: 1 ratio, matched by gender, age ($\pm$ 10 years), and body mass index (BMI, $\pm$ 20%, if possible) in each study part. Subjects in Part A (n = 28) went through three different treatment periods sequentially: single doses of dexpramipexole 75 mg (Treatment 1) and 150 mg (Treatment 2), then 5 doses of 150 mg twice daily (Treatment 3). There was a wash-out period between treatments. Subjects in Part B (n = 28) received dexpramipexole 300 mg every 12 hours for 5 doses. Blood and urine samples were collected pre-dose and intensively post-dose. Safety assessments (adverse events, laboratory tests, vital signs, 12-lead electrocardiogram, physical examination) were conducted during the treatment days, with a final assessment 6 days after last dosing ($\pm$ 2 days).

Results: The time-plasma concentration profile of dexpramipexole was superimposed between Japanese and Caucasian subjects. At the clinical dose of interest (150 mg twice daily), the geometric mean ratio (C_{max}, AUC) of dexpramipexole exposure between Japanese and Caucasian subjects was close to one (90% confidence interval: 0.80, 1.25) after single dose administration and at steady-state. Renal clearance of dexpramipexole was similar in the two ethnic groups; urinary excretion is the primary elimination pathway for dexpramipexole. Dexpramipexole was generally safe and well tolerated in all participants at doses up to 150 mg twice daily. The pharmacokinetic and safety data at 300 mg twice daily (Part B) are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters following single and multiple doses and safety and tolerability of dexpramipexole in healthy Japanese and Caucasian subjects will be reported.

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P21 RECRUITMENT FOR CLINICAL TRIALS INVOLVING FAMILIAL ALS

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Keywords: SOD1, recruitment, clinical trial

Background: Therapies are being developed for SOD1-familial ALS, which represents 1–2% of ALS. Clinical trials involving this relatively rare patient group requires a focus on recruitment of subjects.

Objectives: To assess recruitment strategies used to recruit 32 SOD1 positive familial ALS subjects for Phase I clinical trial involving the antisense oligonucleotide drug Isis-SOD1Rx.

Methods: Recruitment efforts at the national level included 1) Email alerts sent by non-profit organization to distribute information directly to healthcare professionals 2) Contact information was provided for the coordination center for patient inquires. Appropriate patient inquires were then directed to their closest clinical trial site. 3) Athena Diagnostics, a commercial genetic testing company for SOD1, sent a follow-up letter to the ordering physician or clinic with information about the clinical trial. 4) Travel expenses were reimbursed 5) A patient information webinar was presented. 6) The study sponsor implemented a SOD1 genetic testing program for those with a dominant family history of ALS. 7) Trial subjects were allowed to enroll in more than one cohort. 8) Trial sites focused local efforts towards reviewing their current patient populations for eligible subjects, posting information on institutional websites, and speaking at local patient support groups.

Results: Recruitment and enrollment for the first cohort of 8 subjects took over 10 months to complete. Recruitment and enrollment for cohort 2 and cohort 3 took 3 months and recruitment and enrollment for cohort 4 was completed in 2 months. A total of 21 subjects were enrolled as the protocol allowed for subjects to participate in more than one cohort. There were only three screen failures. Total recruitment and enrollment for this trial was completed in 24 months.

Conclusions: Recruitment for this clinical trial was slow initially. Barriers to recruitment and enrollment included initial lack of genetic testing by the trial, the rural location of the patient population, lack of information about available trials for the specific patient population and some difficulty in refining clinical trial sites for enrollment efficiency. Recruitment for later cohorts was faster. As new therapeutic efforts target specific genetic mutations, one area of clinical trial development and strategy should be to develop mechanisms to cultivate and maintain relationships with families affected by rare genetic disorders to enhance and improve clinical trial enrollment. These mechanisms may include involvement in natural history studies, pre-symptomatic familial studies, and enrollment in genetic and/or biomarker databases.

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P22 PROSPECTIVE ASSESSMENT OF CLINICAL TRIAL CHARACTERISTICS THAT MAY AFFECT EFFICIENCIES OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) CLINICAL TRIALS AND PARTICIPATION BY ALS PATIENTS

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Keywords: benchmarking, clinical trial recruitment, clinical trial efficiency

Background: At each clinic visit, ALS patients anxiously request opportunities to participate in clinical studies and therapeutic trials. Despite this interest from patients, only a small number are involved in clinical research and attention to the characteristics of such patients identify that they constitute a group of patients with better disease trajectories. To involve ALS patients in such endeavors requires attention by clinicians and clinical researchers to improving time and financial efficiencies that will allow greater patient participation in research embedded within clinical encounters. There are a number of challenges in store for entering more patients in clinical trials.

Objective: Identify characteristics of ALS and neuromuscular disease clinical trials that prevent efficiencies allowing increased numbers of patients to safely participate in more clinical trials.

Methods: Prospective review of clinical research and clinical trial protocols at Carolinas Medical Center Department of Neurology Research Division bi-weekly meeting including MDs and PhD principal investigators, RN coordinators and non-RN study coordinators including subject recruitment/enrollment, study procedures, subject scheduling, adverse events. ALS studies (12) consisted of three intravenous, one enteral feeding, six oral/enteral feeding and two physical interventions. Neuromuscular studies (6) consisted of one intravenous, two oral/enteral feeding interventions and three natural history collections. A center-based biorepository clinical study collected serum/plasma, PBMC, CSF, tissues samples across ALS, neuromuscular and other neurological diseases. Study difficulties and inefficiencies were defined and tabulated prospectively.

Results: Review identified 1) disparities in inclusion/exclusion criteria, 2) no common data elements, 3) different clinical procedures, 4) redundancy in clinimetrics, 5) differences in definition of adverse events (including or not including disease progression), 6) different allowed comorbidities, 7) differences in drug delivery regimen and involvement of investigational pharmacy services, 8) different types of strength measure, 9) inability to perform study procedures and 10) loss of caregiver in advanced patients. Ongoing budget disparities for patient remuneration for participation and non-standard additional costs to finish a clinical trial were additional financial issues that precluded enrolling more patients in clinical trials.

Conclusions: Ten challenges with respect to entering more patients with ALS into clinical trials were identified. Three of these will be addressed by the National Institutes of Neurological Diseases and Stroke Common Data Elements initiative if it becomes the standard of practice. Participation by patients with advanced ALS and the impact of participation on the caregiver has not been adequately addressed in clinical trials to date. As patients with ALS extend their disease trajectory based on standard of care treatment and interventions, then longer clinical trials and more patients will be required.

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P23 COMPARING SURVIVAL WITH AND WITHOUT DIAPHRAGM PACING (DPS)

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Keywords: respiratory, diaphragm pacer, outcomes

Background: With FDA approval, a number of ALS patients will undergo diaphragm pacing (DPS), with the hope of improving survival in coming years. Support for the procedure is based on a comparison to a control group receiving standard respiratory care, but there has still been no clinical trial comparing survival with versus without DPS.

Objectives: To estimate survival outcomes and understand potential biases among patients that underwent DPS. This was done by comparing them to cohorts obtained from two separate studies; one compared early versus standard NPPV (Lechtzin et al.) and a second with the addition of minocycline.

Methods: We measured survival time starting from initiation of NPPV for each patient and applied Cox proportional hazards models to determine the effects of age, gender, site of onset, FVC, riluzole use, and length of time to NPPV on survival. We adjusted covariates that were significant (at $p < 0.05$) in testing whether survival differed in patients receiving DPS compared with the other cohorts.

Results: Follow-up data were available for 77 patients with DPS and 258 without (190 from the Lechtzin study and 68 placebo from the minocycline trial). We found significant differences among the groups in baseline values for FVC, riluzole usage, age and sex. We found that survival was affected by FVC, age, and time to initiate NPPV following diagnosis. The unadjusted hazard ratio for DPS was 0.37 (95% CI 0.27 to 0.51) and 0.40 (95% CI 0.29 to 0.56) after adjustment for FVC, age and time to initiate NPPV. Each HR is highly significant ($p < 0.001$).

Median survival was 26.4 mos (95% CI 19.6 to 42.5) for DPS, 10.4 mos (95%CI 9.0 to 12.9) for Lechtzin study and 10.1 mos (95% CI 8.3 to 13.6) for minocycline placebo patients.

Discussion: Our findings support the hypothesis that DPS improves survival, even after statistically adjusting for imbalances among the study populations. The risk reduction was estimated to be around 60% and median survival was extended by 16 months. We could not exclude certain biases, however. For example, length-time bias (proactive patients seek earlier treatments thus extending the apparent survival time as measured from treatment initiation) and selection bias (sicker appearing patients are denied surgery and given standard care) are not easily quantitated by these studies. An understanding of these concerns is necessary so that a recommendation for the performance of the surgery is carried forward, while a clinical trial is necessary to rule out other factors.

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P24 PROSPECTIVE STUDY OF RADIOTHERAPY OF SALIVARY GLANDS AS TREATMENT OF SIALORRHEA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: radiotherapy, salivary glands, sialorrhea

Background: Sialorrhea is a frequent and disabling symptom in patients with amyotrophic lateral sclerosis (ALS). Medical treatment is often poorly effective and/or not well-tolerated. Radiotherapy of salivary glands can be an interesting therapeutic option.

Objective: Estimate efficiency and tolerance of radiotherapy of salivary glands in patients with ALS.

Methods: Prospective monocentric study in ALS patients with sialorrhea treated by radiotherapy in the Clinique De La Porte De Saint Cloud (Boulogne-Billancourt, France). Preliminary results of 40 patients treated between November 2010 and November 2011 are presented. All patients had conformational radiotherapy. Total dose was 10 Gy in 2 fractions on 3 days in 27 patients and 20 Gy in 4 fractions on 10 days in 13 patients. We used two 6 MV photon opposed beams and radiation volume including both submaxillary glands and the two thirds of both parotid glands. Patients had clinical examination at the end of treatment, 1 month and 3 months later. Efficiency of radiotherapy was evaluated with the 9-grades Sialorrhea Scoring Scale.

Results: 32 complete responses (SSS 1, 2) and 8 partial responses (SSS 3, 4, 5) were observed at the end of the treatment. Follow-up at 1 month and 3 months showed a complete response in 16 and 11 patients, respectively, and a partial response in 16 and five patients. Treatment was well tolerated. Acute toxicity was observed in 15 patients : xerostomia in three patients, taste modification in five patients, pharyngeal pain in four patients and thick saliva in three patients. All side effects were resolved in the days following the end of the treatment. Seven patients treated at dose of 10 Gy relapsed and had a second radiotherapy at the same dose with good results.

Discussion: Radiotherapy allowed a dramatic decrease or resolution of sialorrhea. Inter-individual variability explains the relapse in some patients and the dose of 10 Gy is probably not sufficient to maintain a long response. Treatment is well tolerated and toxicity is low with both doses of 10 and 20 Gy. Side effects are transitory even in patients who have a second radiotherapy.

Conclusion: Radiotherapy of salivary glands in ALS patients with sialorrhea appears as a very interesting therapeutic option. A larger number of patients and a longer follow-up remain necessary to confirm these encouraging preliminary results.

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P25 POSTERIOR PHARYNGEAL AUGMENTATION BY AUTOLOGOUS LIPOINJECTION FOR DYSARTHRIA IN ALS

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Keywords: pharyngeal augmentation, dysarthria, velopharyngeal incompetence

Background: Dysarthria is a motor disorder of articulation, phonation, and respiration, resulting in unintelligibility of speech. In ALS, progressive paresis of bulbar muscles causes velopharyngeal incompetence (VPI) with advancing communication problems. Guidelines recommend alternative and augmentative communication devices. To reduce hypernasality, a nose peg often is uncomfortable and not well accepted by patients. Furthermore, a palatal lift or augmentation prosthesis can be helpful. However, it might be impossible to adapt or wear the prosthesis because of a hyperactive gag reflex or dysphagia. In other conditions of VPI, various surgical management options exist: augmentation of the posterior pharyngeal wall or the soft palate by autologous lipoinjection, is minimally invasive, and has shown effective, lasting results for restoring normal resonance. Therefore, it could also be applied in ALS patients.

Objectives: To report on two patients with ALS who underwent autologous fat injection of the posterior pharyngeal wall for VPI.

Methods: Patients were examined interprofessionally by experts of ALS clinics (a neurologist, an otorhinolaryngologist, a speech pathologist) pre- and postoperatively. Videoendoscopic evaluation of velar function with videographic documentation was made before and two months after surgical treatment. Under general anesthesia, autologous abdominal fat was aspired, centrifugated, and afterwards injected into the posterior pharyngeal wall. Postoperatively, the patients had to wear a flexible abdominal belt for 5 days.

Results: In 2010 and 2011, posterior pharyngeal augmentation was recommended to two male patients with upper-motoneuron-predominant ALS and marked rhinolalia aperta. As gag reflex was clearly increased, palatal prosthesis was not suggested. At the time of operation, the patients were aged 56 and 54 years and had an ALS-FRS-R of 29/48 and 27/48, respectively. Both patients had only mild dysphagia and were orally fed. The disease was characterized by bulbar- and lower-limb-onset, a duration of 7 and 3 years, respectively. Improvement in articulation was demonstrated in both cases postoperatively, and both patients reported that speaking was less fatiguing. As in the 54-year old patient intelligibility of speech deteriorated again, autologous fat grafting was re-applied 7 months later, resulting in improved intelligibility again. Each operation was well tolerated without any side effects.

Discussion: In the field of plastic surgery, fat grafts have become an important procedure with proven long term survival. However, in ALS autologous fat injection of the posterior pharyngeal wall has not been described up to now. Furthermore, distribution and evolution of symptoms substantially differ in ALS patients. Th[illegible]

potential benefits, risks, and costs of lipoinjection for VPI are crucial factors.

Conclusions: Dysarthria in ALS remains a significant problem with limited therapeutic options. Augmentation of the posterior pharyngeal wall by autologous fat grafting was a safe and effective treatment in two ALS patients with VPI, and should be considered in selected patients with ALS.

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P26 THE ROLE OF EXERCISE AND ITS IMPACT ON DISEASE PROGRESSION AND SEVERITY IN ALS

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Keywords: human, exercise, survival

Background: Controversy exists over the possible benefits of physical therapy and exercise as a therapeutic tool to slow disease progression and improve quality of life in ALS. Some studies suggest increased functional ability and reduced disease progression with exercise while others suggest that vigorous physical activity can aggravate excitotoxicity, oxidative stress and increase calcium loads causing selective degeneration of vulnerable motor neurons. Additionally, animal studies suggest that swimming-based therapy may be beneficial (1); however, it is unclear if a specific exercise is more beneficial in ALS patients.

Objectives: To evaluate the impact of exercise in a large cohort of ALS patients with attention to different types of exercise.

Methods: Information from consecutive patients initially evaluated at Hospital for Special Care's (HSC) ALS Clinic between 2007 and 2010 was collected retrospectively. Patients were divided into two groups: those who reported any exercise beyond activities of daily living and those who did not do any additional exercise. The exercise group was further subdivided into types of exercise performed: aerobic, aqua therapy, resistance, and other exercise. Survival, forced vital capacity(FVC) and ALS functional rating scores(ALSFRS) were collected as endpoint measures. Analysis of the data included single factor ANOVA of survival between different types of exercise with confidence intervals. Comparison of means was done with a two sample, t-test and comparison of proportions wasperformed using chi-square test.

Results: Of the 234 ALS patients, 56.8% were male, mean age of disease onset was 63.8 + /-12.6 yrs, 29% were on riluzole, mean initial FVC was 83% + /- 27%, and the initial region of onset was: bulbar(27%), cervical(33%), lumbosacral(37%), thoracic(2%), and respiratory(1%). 27%(62/234) reported exercise: 40.3% aqua therapy, 30.7% aerobic, 9.7% resistance, and 19.4% other. A single factor ANOVA analysis showed no difference in ALS disease duration for different modes of exercise ($p = 0.6867$). 95% confidence intervals showed that aqua therapy promised most with average disease duration between 27.9 and 51.6 months compared to no exercise which showed 95% confidence interval of disease duration between 28.2 and 37.3 months. Although there was not sufficient evidence ($p = 0.1339$), a trend was noted for increased survival in ALS patients who did aqua therapy compared to those who did not. Also, a trend of slower rate of decline inALSFRS (-1.08vs.-1.51,$p = 0.0759$) and lung function (-1.88%vs.-3.36%,$p = 0.0895$) was noted in the exercise group vs. no exercise group.

Discussion and conclusions: There was no significant difference in survival between exercise and no exercise groups suggesting no detrimental effect of exercise in ALS. A trend of improved survival with aqua therapy in ALS patients was noted and is consistent with studies done in ALS mouse models where swimming-based training sustained motor function and increased survival(1).

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THEME 2 IN VIVO EXPERIMENTAL MODELS

P27 THE EFFECT OF TREADMILL EXERCISE AND METALLOTHIONEIN TREATMENT ON SURVIVAL AND FUNCTIONAL OUTCOMES OF THE SOD1-G93A MOUSE MODEL
K Lewis, L Blizzard, W Bennett, RS Chung, MI Chuah

P28 MOLECULAR MECHANISMS OF GOLGI PATHOLOGY IN PROGRESSIVE MOTOR NEURONOPATHY
S Bellouze, M Schäfer, C Rabouille, G Haase

P29 MODELLING SITE SPECIFIC EXCITOTOXICITY: REVEALING MECHANISMS OF AMYOTROPHIC LATERAL SCLEROSIS
C Blizzard, K Southam, A King, T Dickson

P30 TARGETTING GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN EXPERIMENTAL ALS
M Milanese, T Bonifacino, A Puliti, M Melone, I Mustante, F Giribaldi, A Voci, F Conti, G Bonanno

P31 ACTIVE ZONE LOSS IN NEUROMUSCULAR JUNCTIONS OF AMYOTROPHIC LATERAL SCLEROSIS RODENTS
H Nishimune, J Chen, L Nadeau, T Numata, Y Aoki, Y Mori

P32 PROGRESSIVE AND SELECTIVE DEGENERATION OF GENETICALLY LABELED CORTICOSPINAL MOTOR NEURONS AND RELATED SUBCEREBRAL PROJECTION NEURONS IN HSOD1G93A-UEGFP MICE
M Yasvoina, B Genc, J Jara, P Sheets, N Yang, A Milosevic, G Shepherd, H Ozdinler

P33 VISUALIZATION AND CHARACTERIZATION OF CELL-TYPE SPECIFIC NEUROINFLAMMATORY COMPONENTS IN ALS USING MCP1-CCR2-HSOD1G93A TRIPLE TRANSGENIC MICE
J Jara, RG Miller, H Ozdinler

P34 REGIONAL HETEROGENEITY OF IN VIVO ASTROGLIA TRANSCRIPTOME AND PATHOLOGICAL ALTERATIONS OF NATIVE ASTROGLIA IN SOD1 MOUSE MODEL
Y-C Hsieh, P-W Zhang, C Donnelly, R Sattler, J Rothstein

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THEME 2 *IN VIVO* EXPERIMENTAL MODELS

P27 THE EFFECT OF TREADMILL EXERCISE AND METALLOTHIONEIN TREATMENT ON SURVIVAL AND FUNCTIONAL OUTCOMES OF THE SOD1-G93A MOUSE MODEL

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Keywords: metallothionein, exercise, linear mixed modelling

Background: A number of studies in the SOD1-G93A mouse model suggest that exercise can increase survival time and delay disease progression; however other studies have found that exercise provides no benefit. Metallothionein-I/II (MT-I/II) is a highly conserved antioxidant protein, which has demonstrated neuroprotective properties both *in vitro* and *in vivo*. SOD1-G93A mice display a worsened disease phenotype with decreased survival when MT-I/II is genetically ablated. MT-I/II levels in ALS patient spinal cord are reported to be altered, although it is unclear whether they are increased or decreased. Exercise can increase the MT-I/II levels in wild-type mouse spinal cord.

Objectives: Study 1: We examined the effect of treadmill running exercise and exogenous MT-I/II administration on survival and functional parameters in the SOD1-G93A mouse model. Study 2: We examined the effect of emtins, synthetic peptides based on the MT-I/II sequence, on these outcome measures.

Methods: Study 1: Mice received a combination of MT-I/II treatment and exercise treatment from 6-16 weeks of age - either MT-I/II protein at 20mg/kg/wk, or saline control; and also either treadmill running exercise at 10m/min, 30min/day, 5day/wk, or no exercise. Three mice/group were then sacrificed for histology, and 10mice/group were followed until disease endpoint. Study 2: Mice received EmtinA or EmtinB (30mg/kg/wk) or vehicle control, from 14 weeks of age until disease endpoint. We examined survival time, body weight and stride length.

Results: In both studies, no significant differences in survival were found using Kaplan-Meier analysis. However, using Cox regression we estimated the effects of two individual factors (MT-I/II and exercise) on survival. Cox regression hazard rates were lower for those receiving MT-I/II than those receiving saline but were not statistically different between exercise-treated and sedentary mice. Using mixed modeling, no significant differences were found in body weight between mice receiving either emtins or vehicle. However, in mice receiving a mix of MT-I/II and exercise treatments, mixed modeling revealed that the slope coefficients for MT-I/II treatment were significantly different from vehicle control treatment. These differences suggest that mice receiving MT-I/II had a higher maximum body weight and later age at maximum body weight, than vehicle controls. No significant differences were found between sedentary and exercised mice. Emtin treatment appeared to increase survival and maintain stride length, but these results were not statistically significant, possibly due to the small sample size in this study.

Discussion and conclusions: Survival appears to be affected by MT-I/II treatment but not by exercise. Stride length was not significantly affected by MT-I/II or emtin treatment, but showed subtle changes which we are investigating with mixed modelling. We are also performing spinal cord and muscle histology on tissue from MT-I/II- and exercise-treated mice, to determine whether MT-I/II increases survival by protecting motor neurons or maintaining neuromuscular junctions.

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P28 MOLECULAR MECHANISMS OF GOLGI PATHOLOGY IN PROGRESSIVE MOTOR NEURONOPATHY

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Keywords: Golgi apparatus, vesicle traffic, SNARE

Pathology of the Golgi apparatus represents one of the earliest features of degenerating motor neurons in amyotrophic lateral sclerosis (ALS) and related diseases but its molecular causes and mechanisms remain unclear. To investigate the potential role of microtubule defects and protein aggregates in ALS-linked Golgi pathology, we studied *pmn* mice with progressive motor neuronopathy which are mutated in the tubulin chaperone TBCE (1,2).

Here we demonstrate severe progressive Golgi fragmentation and atrophy in motor neurons of *pmn* mice by using immunofluorescence analyses, 3D organelle modeling and electron microscopy. During disease progression, Golgi cisternae were progressively transformed into a convolute of small vesicles. In parallel, the Golgi v- (vesicular) SNARE proteins GS15 and GS28 were drastically up-regulated while their corresponding t- (target-) SNARE protein Syntaxin-5 was present at normal levels. Golgi pathology in *pmn* motor neurons was completely rescued by transgenic wildtype TBCE but not mimicked by nerve axotomy indicating loss of TBCE function as its origin. The distinct effects of TBCE depletion, folding-deficient tubulin mutants and pharmacological microtubule disruption on Golgi structure in cultured motor neurons identified loss of microtubules rather than accumulation of misfolded tubulin proteins as causative for Golgi pathology. Defective microtubule growth at Golgi membranes was shown to impede the traffic of Golgi-derived

vesicles leading to their decreased docking and fusion at target membranes.

To our knowledge these data provide the first mechanistic explanation for Golgi pathology in motor neuron disease.

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P29 MODELLING SITE SPECIFIC EXCITOTOXICITY: REVEALING MECHANISMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: excitotoxicity, axon degeneration, primary culture

Background: It is now accepted in a number of neurodegenerative diseases that axon degeneration can occur independently of apoptosis, but it is not known how the cell body is involved in axon 'die back'. We have shown that chronic low levels of kainic acid can cause a distal axonopathy in cultured motor neurons that shows strong similarities, both morphologicaly and immunohistochemically, to human ALS pathology (1).

Objective: To investigate the primary site of excitotoxic damage and the mechanism of ALS-like functional decline we have developed two complimentary models of site-specific excitotoxicity. This approach is particularly significant for ALS where neuronal compartments are spatially separated in the cortex, spinal cord, nerves and muscle.

Methods: *In vitro:* Spinal motor neurons were grown in microfluidic chambers supported by primary glia and skeletal muscle which results in fluidic separation of the soma, axon and neuromuscular junction (NMJ) compartments. Mature (21 DIV) motor neurons ($n = 5$) were exposed to kainic acid (100um,1-24hrs). *In vivo:* Osmotic mini pumps were implanted in C57/Bl6 mice to deliver a chronic infusion of either (i) kainic acid (1-5mM) to the subarachnoid space of the lumbar region (L4-5) or (ii) glutamate (5mM) to the gastrocnemius muscle. The anterograde tracer fluoro ruby (2uM) was concomitantly infused to both sites.

Results: *In vitro,* axonal swellings were rarely present in untreated cultures. At 6h of KA (100um) exposure there was a significant ($P < 0.001$) increase in the number of swollen axon distal segments (16.6 ± 3.2 per coverslip) relative to untreated cultures (0.75 ± 0.19). Putative neuromuscular junctions were present in motor neuron-skeletal muscle co-cultures, indicated by clustering of acetylcholine receptors. *In vivo*, 28 days after implantation, Fluoro Ruby labelling was present throughout cells within the subarachnoid space (L4-L6) and a small number of cells within the ventral horn, indicating a targeted delivery of KA. Immunolabelling revealed that these cells were motor neurons. Gastrocnemius muscles of the KA treated and vehicle control treated mice were double labelled for synaptophysin and alpha-bungarotoxin and NMJ synapses were graded as either intact or degenerating. There was a significant increase in the percentage of degenerating synapses in the treated vs control mice, but an increase in the number of branch points ineach junction, indicating the compensatory remodelling and plasticity may be an early event in axonal pathogenesis.

Conclusion: By combining the high throughput *in vivo* culture system with the complimentary sophisticated *in vivo* model we are uniquely placed to reveal critical elements of the ALS disease mechanism and sequence of disease progression and to rapidly screen appropriate novel therapeutic agents specifically directed to the affected neuronal compartment.

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P30 TARGETTING GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN EXPERIMENTAL ALS

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Keywords: group 1 metabotrophic glutamate receptors, glutamate release and excitotoxicity, genetic mouse models

Background: Glutamate(Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (MNs) in ALS and reduced astrocytary transport, which in turn increases the synaptic availability of Glu, was suggested as a cause (1). On the basis of our studies, we have proposed that abnormal release may well be another source of excessive Glu and that altered Glu release mechanisms may represent possible targets for therapeutic approaches (2,3).

Our previous results have demonstrated the existence of excessive increase of Glu release in SOD1/G93A mice mediated by Group I (mGlu1 and mGlu5) receptor activation (4), which may represent a cause of excessive Glu and of neurodegeneration.

Objectives: To prove the impact of Group I metabotropic glutamate receptor blockade in experimental ALS.

Methods: To provide a genetic tool to evaluate the role of mGlu1 receptors in ALS, we generated mice carrying half expression of mGlu1 receptors in the SOD1/G93A background, by crossing ALS mutant mice with $Grm1^{+/crv4}$ mice, lacking mGlu1 receptors because of a spontaneous recessive mutation. In the same line we also generated mice carrying half expression of mGlu5 receptors, by crossing ALS mutant mice with Grm5 −/+ mice. Survival, motor abilities, histology for MNs and mitocondrial damage, oxidative stress markers, biochemistry for astrogliosys and microglia activation, receptor expression, glutamate release were investigated to assess the phenotype modifications in double mutants respect to SOD1/G93A mice.

Results: mGlu 1 receptor deficient double mutants showed prolonged survival probability respect to single mutant SOD1/G93A mice. Accordingly, slower disea

and improved motor performances were observed. Histological studies showed higher number of Chat-positive MNs in spinal cord, reduced axonal degeneration and mitocondrial damage and reduced astrocyte and microglia activation in double mutant mice at a late phase of the disease progression. Metallothioneins and glutathione S-transferase were studied as oxidative stress markers. The up-regulation of metallothionein and glutathione-S-transferase mRNA expression, observed in SOD1/G93A mice, was largely normalized in double mutants lacking of mGlu1 receptors. Over expression of mGlu5 receptors and Group I metabotropic glutamate receptor-induced abnormal release of Glu, observed in SOD1/G93A mice, were reduced in double mutant animals. Also mGlu5 receptor-lacking SOD1/G93A mice showed remarkable prolonged survival and phenotype amelioration.

Discussion and conclusions: mGlu1 or mGlu5 receptor deletion has a significant impact in-vivo on SOD1/G93A mice pathology. These results would provide the rationale for pharmacological approaches to ALS by selectively blocking Group I metabotropic Glu receptors. With this aim pharmacological treatments with Group I receptor antagonists are in progress.

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P31 ACTIVE ZONE LOSS IN NEUROMUSCULAR JUNCTIONS OF AMYOTROPHIC LATERAL SCLEROSIS RODENTS

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Keywords: Bassoon, calcium channels, denervation

Background: In amyotrophic lateral sclerosis (ALS), denervation of neuromuscular junctions (NMJs) precedes the motor neuron degeneration, suggesting that ALS is a distal axonopathy. However, the mechanism of presymptomatic denervation and the etiology of ALS remain unknown.

Objectives: To analyze NMJ active zones of SOD1^{G93A} mice for a better understating of the distal axonopathy.

Methods: The time course of NMJ denervation in diaphragms of SOD1^{G93A} mice was analyzed by immunohistochemistry from postnatal day (P) 27 to P130. NMJ active zones were analyzed by transmission electron microscopy and immunohistochemistry detecting active zone protein Bassoon. Patch-clamp recordings were used to analyze the Ca^{2+} influx through P/Q-type VDCCs interacting with Bassoon.

Results: Diaphragm NMJs of SOD1^{G93A} mice showed denervation in the presymptomatic stage of P85. Next, we analyzed presynaptic terminals and active zones because denervation may be caused by an impairment of presynaptic functions or active zones—the synaptic vesicle release sites. Decreased number of active zones in innervated NMJs of SOD1^{G93A} mice at P85 was confirmed by electron microscopy and Bassoon immunohistochemistry. Furthermore, similar active zone defect was confirmed in SOD1^{G93A} rats. The active zone defects did not seem to be downstream of mitochondria defects in motor neurons because the decrease of active zone number was as severe in presynaptic terminals with relatively normal mitochondria as in presynaptic terminals with degenerated mitochondria/vacuoles. These active zone impairments are likely to weaken the presynaptic function in NMJs because we recently discovered (a) the binding between Bassoon and P/Q-type VDCCs, and (b) the role of P/Q-type VDCC as a scaffolding protein in active zone organization at NMJs. Furthermore, we now show that the interaction between P/Q-type VDCCs and Bassoon augmented the Ca^{2+} influx through the VDCC, suggesting that the Ca^{2+} influx may be attenuated by the loss of Bassoon at ALS NMJs.

Discussion: The role of Bassoon in VDCC modulation and the known role of Bassoon in synaptic vesicle trafficking suggest that synaptic transmission may be attenuated in NMJs lacking Bassoon. Thus, the loss of Bassoon from ALS NMJs may be a part of the etiology of presymptomatic denervation.

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P32 PROGRESSIVE AND SELECTIVE DEGENERATION OF GENETICALLY LABELED CORTICOSPINAL MOTOR NEURONS AND RELATED SUBCEREBRAL PROJECTION NEURONS IN HSOD1G93A-UEGFP MICE

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Keywords: reporter mouse, SOD1, motor neuron

Background: Understanding mechanisms that control motor neuron vulnerability and degeneration require visualization, identification, isolation and detailed cellular analysis of affected neuron populations at different stages of disease initiation and progression. Therefore, to understand mechanisms that control corticospinal motor neuron (CSMN) and spinal motor neuron vulnerability, it is important to develop tools that allow visualization and isolation of both cortical and spinal components of motor neuron circuitry in wild type mice and mouse models of neurodegenerative disorders.

Objectives: To confirm CSMN identity of eGFP + neurons in the motor cortex of UCHL1-eGFP mouse and to investigate whether hSOD1^{G93A}-UeGFP transgenic ALS mouse model would serve as an ALS reporter line by recapitulating the previously reported CSMN/SCPN degeneration in the hSOD1^{G93A} mouse.

Methods: CSMN identity eGFP + neurons in layer 5 of the motor cortex are confirmed by anatomy, axon projection path, retrograde labeling, molecular marker expression, electrophysiological analysis and cortical circuit mapping. hSOD1^{G93A}-UeGFP mouse lines are generated by crossbreeding hSOD1^{G93A}

transgenic ALS mice with UCHL1-eGFP reporter mice. CSMN and subcerebral projection neuron (SCPN) degeneration is examined and quantitatively measured at P30, P60, P90 and P120 (n = 3 mice/time point).

Results: CSMN in layer 5 of the motor cortex and SCPN located outside of the motor cortex are genetically labeled by eGFP expression between P0 and P365. CSMN degeneration in the motor cortex and SCPN loss in the somatosensory cortex in the hSOD1^{G93A}-UeGFP mouse closely correlate with the well-established neuron loss in the hSOD1^{G93A} mouse. This further confirms motor neuron identity of eGFP + neurons in the motor cortex. Our findings also reveal progressive CSMN/SCPN degeneration in hSOD1^{G93A}-UeGFP mouse, confirming its potential use as an ALS disease reporter line to study the biology of CSMN with respect to disease pathology.

Discussion: This novel reporter line will now allow cell type-specific visualization, purification and analysis of CSMN with respect to motor neuron circuitry degeneration in models of ALS and other motor neuron disorders.

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P33 VISUALIZATION AND CHARACTERIZATION OF CELL-TYPE SPECIFIC NEUROINFLAMMATORY COMPONENTS IN ALS USING MCP1-CCR2-HSOD1G93A TRIPLE TRANSGENIC MICE

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Keywords: neuroinflammation, MCP1, CCR2

Background: Innate and adaptive immune response plays a major, but not fully elucidated role in ALS pathology. Microglial activation and astrogliosis are major components of the neuroinflammation observed in the spinal cord and motor cortex of ALS patients as well as ALS animal models. During disease progression non-neuronal cells play a key, yet completely undefined, role in neuronal susceptibility by their cytokine and receptor expression. The involvement of cytokine/receptor MCP1 (monocyte chemoattractant protein-1, aka CCL2)/ CCR2 (CC chemokine receptor 2) has been demonstrated in both ALS patients and ALS animal models. Increased MCP1 plasmatic levels in association with activation of the innate immune response in ALS patients are linked to decreased plasmatic levels of CCR2 indicating their relevance during disease progression.

Objectives: To visualize and isolate cellular components of the innate and adaptive immune response, and to investigate key players of neuroinflammation in a cell-type specific manner in the hSOD1^{G93A} ALS transgenic mouse model.

Methods: Generation of MCP1-CCR2-hSOD1^{G93A} triple transgenic mice where MCP1 and CCR2 are genetically labeled with mRFP (monomeric red fluorescent protein 1) and EGFP (enhanced green fluorescent protein), respectively, in the hSOD1^{G93A} ALS transgenic mouse model. Using immunocytochemistry approaches we evaluated the identity of MCP1 + and CCR2 + cells using markers specific for microglia, astrocyte, dendritic cell, and infiltrating monocyte cells at presymptomatic age (P30), early symptomatic (P60), symptomatic (P90), and end-stage (P120).

Results: Our results indicate the presence of MCP1 + and CCR2 + cells at multiple stages during disease initiation and progression. Their relative location to vulnerable motor neurons both in the cortex and spinal cord, meninges, corticospinal tract, peripheral vasculature, together with their cellular morphology and increased numbers strongly implicate their involvement in pathology even at asymptomatic disease stage (P30). Isolation MCP1 + and CCR2 + cells from motor cortex, cervical and lumbar spinal cord by fluorescence-activated cell sorting (FACS) approaches at P30 demonstrate the feasibility of identifying cellular components responsible for the initiation and progression of innate and adaptive immune response.

Discussion: Since multiple cytokines and neuroinflammation components are present at different levels during ALS progression, it is critical to analyze components individually to dissect out their possible role. Our studies allow direct visualization and isolation of MCP1 + and CCR2 + cells with respect to disease initiation and progression and their cell type specific analysis will provide valuable information regarding their microglia and astrocyte identity in the motor cortex.

Conclusions: In this study we identified MCP1 + and CCR2 + cell profile during disease progression in ALS. Future analysis of MCP1 + and CCR2 + cells will help elucidate cellular mechanisms in motor neuron vulnerability as well as possible drug targets.

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P34 REGIONAL HETEROGENEITY OF *IN VIVO* ASTROGLIA TRANSCRIPTOME AND PATHOLOGICAL ALTERATIONS OF NATIVE ASTROGLIA IN SOD1 MOUSE MODEL

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Keywords: astroglia, transcriptome, BAC transgenic mice

Background: Approximately 10% of ALS shows a familial inheritance, and 10%–20% of these cases are caused by mutations of the Cu/Zn superoxide dismutase (SOD1). A toxic gain of function of human SOD1 causes pronounced motor deficits *in vivo* correlated to motor neuron degeneration in a number of animal models, but molecular mechanisms underlying this pathology remain unknown. Astrocytes, the most abundant cells in the CNS, are activated in neurodegenerative processes and have a significant impact on motor neuron survival. Furthermore, evidence has accumulated suggesting heterogeneity of astrocytes across different brain regions and the spinal cord.

Methods: We used FACS (fluorescent-activated cell sorting) to acutely isolate adult astrocytes from BAC transgenic mice that express enhanced green fluorescent protein (EGFP) under the control of GFAP, ALDH1L1 and GLT1-promoters. Microarrays analyses were performed with RNA samples of FAC sorted astrocytes obtained from BAC mice

crossed with the SOD1G93A animals and non-transgenic littermates.

Results: In the pre-symptomatic stage, the molecular gene profiles of ALDH1L1-EGFP +, GFAP-EGFP + and GLT1-EGFP + cells are similar in the CNS. At end stage, the number of GLT1-EGFP + cells obtained from spinal cord is dramatically reduced compared to ALDH1L1-EGFP + and GFAP-EGFP + cells and thus only ALDH1L1-EGFP + and GFAP-EGFP + cells were used for end stage analysis. Our analysis revealed that adult astroglial gene expression was altered for numerous classes of transcripts, including membrane transporters or channels (eg connexin 43, 30; GLT1), energy metabolism (eg GPD2) and transcription factors (eg TRPS1) in spinal cord or brains of end stage animals. Our control molecular profiling data of *in vivo* glial cells also suggests that astrocytes are clearly heterogeneous with regard to their gene expression profiles in forebrain, hindbrain and spinal cord.

Discussion and conclusions: The expression of numerous genes has been altered in spinal cord or brains at the end stage of SOD1 animals. The pathological alteration of astrocyte-specific genes may contribute to the identification of gene candidates for cell type-specific therapies in ALS.

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P35 GENETIC, CELL-TYPE SPECIFIC LABELING IN HSOD1G93A-UEGFP MICE REVEALS PROGRESSIVE DEGENERATION OF SPINAL MOTOR NEURONS

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Keywords: motor neuron, SOD1, reporter

Background: Understanding mechanisms that control motor neuron vulnerability and degeneration require visualization and detailed cellular analysis of affected neurons at different stages of disease. Reporter mouse lines expressing green fluorescent protein (GFP) gene in a subset of neurons has been revolutionary in understanding biology of labeled neuron populations.

Objectives: To develop tools that allow visualization and isolation of motor neurons circuitry in wild type mice and mouse models of neurodegenerative disorders.

Methods: UCHL1-eGFP reporter mice have been generated using a BAC construct, driving the eGFP expression under the control of the UCHL1 promoter. eGFP + neurons have been characterized using anatomical, molecular marker expression and electrophysiological analysis. hSOD1^{G93A}-UeGFP mouse has been generated by crossbreeding hSOD1^{G93A} transgenic ALS mice with UCHL1-eGFP reporter mice. SMN degeneration is studied and quantitatively measured at P30, P60, P90 and P120 (n = 3 mice/time point).

Results: We generated UCHL1-eGFP and hSOD1^{G93A}-UeGFP mouse lines, in which spinal motor neurons (SMN) and peripheral neurons are genetically labeled by eGFP expression between P0 and P365. SMN identity of eGFP + neurons is confirmed by molecular marker expression, presence of eGFP + axons in the neuromuscular junctions (NMJ) and electrophysiological analysis. SMN degeneration in the hSOD1^{G93A}-UeGFP mouse closely correlates the well-established spatial and temporal motor neuron degeneration in the hSOD1^{G93A} mouse. Likewise, NMJ defects in the hSOD1^{G93A}-UeGFP mouse are in line with previously published findings in the hSOD1^{G93A} mouse.

Discussion: This novel reporter line will now allow cell type-specific visualization, purification and detailed cellular analysis of SMN and peripheral neurons.

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P36 AUTOPHAGY IN THE SPINAL MOTOR NEURONS OF CONDITIONAL ADAR2-KNOCKOUT MICE

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Keywords: ADAR2-knockout mice, autophagy, ultrastructure

Background: There have been some reports indicating that AMPA receptor-mediated excitotoxic mechanism plays a pathogenic role in ALS and SOD1-associated familial ALS model animals. Conditional ADAR2-knockout mice demonstrate that the loss of ADAR2 activity induces the slow death of motor neurons, and are considered to be useful to research on sporadic ALS. However, motor neurons of spinal cords have not been studied in relation to autophagy in ADAR2-knockout mice.

Objectives: To clarify if autophagy is involved in the pathomechanism of motor neuron degeneration in this model mouse.

Methods: We studied, immunohistochemically using a rabbit polyclonal ani-LC3 antibody, and electron-microscopically, the motor neurons of cervical spinal cords in homozygous ADAR2$^{flox/flox}$/VAChT-Cre.Fast (AR2) mice (15 wks, n = 2), homozygous ADAR2$^{flox/flox}$/VAChT-Cre.Slow (AR2Slow) mice (140 wks, n = 2), heterozygous ADAR2$^{flox/+}$/VAChT-Cre.Fast (AR2H) mice (14 wks, 17 wks, 74 wks, n = 2, respectively) and age-matched littermates as controls (12 wks, 15 wks, 16 wks, 74 wks, 140 wks, n = 2, respectively). Western blot analyses were performed in anterior horns of the spinal cords of AR2 mice and age-matched control mice (15 wks) (n = 3 for each genotype).

Results: In AR2 mice (15 wks), the cytoplasm of some normal-appearing and degenerated anterior horn neurons was positively immunostained for LC3, and in AR2H mice (74 wks), in addition to the positive-immunoreactivity of the somata of some anterior horn neurons with anti-LC3 antibody, the cytoplasm and perivacuolar regions of motor neurons frequently showed immunopositivity for p62. Western blot analyses showed increased expression of autophagy-associated protein such as Atg5, Atg7 and LC3-II in the anterior horn of AR2 mice (15 wks) as compared with age-matched control mice. Electron-microscopically, in

homozygous and heterozygous ADAR2-knockout mice, autophagosomes surrounded by a double-membrane and autolysosomes surrounded by a single membrane were not uncommonly observed in the cytoplasm of motor neurons. Autophagy was more frequently observed at early stages than at late stages in these mice. Cytoplasmic organelles sequestered by autophagy contained various kinds of materials such as mitochondria, ribosome-like structures, vesicles, electron-dense amorphous materials, and multilamellar bodies. In contrast, autophagosomes were only occasionally observed in the somata of anterior horn neurons and in the myelinated axons in control mice.

Conclusions: Autophagy is significantly activated and upregulated in the cytoplasm of motor neurons, probably due to activated cytosolic Ca^{2+} through increased Ca^{2+} permeability, and may be involved in the pathomechanism of motor neuron degeneration in conditional ADAR2-knockout mice.

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P37 AGING AND ADAR2 ACTIVITY IN MOTOR NEURONS

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Keywords: aging, TDP-43, RNA editing

Objectives: One of the disease-specific and neuronal death-causing molecular abnormality found in motor neurons of patients with sporadic amyotrophic lateral sclerosis (ALS) is an expression of Q/R site-unedited GluA2, an AMPA receptor subunit. This molecular change is due to the downregulation of an RNA-editing enzyme called adenosine deaminase acting on RNA 2 (ADAR2). TDP-43 pathology, a neuropathological hallmark of sporadic ALS, has recently been shown to be closely associated with the downregulation of ADAR2 in the motor neurons of patients with sporadic ALS (1). Because TDP-43 pathology is found more frequently in the brains of elderly patients, we investigated the age-related changes in the localization of TDP-43 and ADAR2 activity in mouse motor neurons.

Methods: Spinal cords of C57BL/6J mice with various ages (raging from 1 day to 104 weeks) were used. Immunohistochemistry for TDP-43 and ADAR2 was performed on the spinal cord sections. Expression level of ADAR2 mRNA in the spinal cord was investigated by means of quantitative RT-PCR. Extents of the editing efficiency at the ADAR2-specific RNA editing sites, including the Q/R site of GluA2 mRNA and the K/E site of cytoplasmic flagile X mental retardation protein interacting protein 2 (CYFIP2) mRNA, were investigated by quantitative measurement of restriction enzyme-dependent fragments of the RT-PCR products in the spinal anterior horn and laser-caputured motor neuron tissues.

Results: We found that the ADAR2 activity was developmentally upregulated, and its mRNA expression level was progressively decreased in the spinal cords of aged mice. Wild-type motor neurons exhibited immunoreacitivity to both ADAR2 and TDP-43 in the nucleus, whereas laterally localized fast fatigable motor neurons, but not centrally localized neurons, in aged mice demonstrated a loss of ADAR2 and abnormal TDP-43 localization. Importantly, these motor neurons expressed significant amounts of the Q/R site-unedited GluA2 mRNA, which is a lethality-causing molecular abnormality observed in the motor neurones of sporadic ALS patients.

Conclusion: These results suggest that an age-related decrease in ADAR2 activity plays a mechanistic role in an age-associated increase of incidence and acceleration of progression of ALS.

Reference

1. Aizawa *et al.* Acta Neuropathol. 2010;120:75–84.

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P38 EXPRESSION OF WILD-TYPE HUMAN SUPEROXIDE DISMUTASE-1 IN MICE CAUSES ALS

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Keywords: wild type, human SOD1, mouse

Background: A common cause of ALS is mutations in the gene encoding superoxide dismutase-1 (SOD1). In several other neurodegenerative conditions some of the proteins found mutated in families are thought to be involved in the pathogenesis also in cases lacking mutations. Could this also pertain to ALS? There is evolving circumstantial evidence suggesting that the wild-type SOD1 can be neurotoxic and more generally involved in the pathogenesis of ALS.

Objectives: To directly test the proposition that wild-type human SOD1 has the ability to cause ALS.

Methods: Mice that express wild-type human SOD1 at a rate close to that of mutant human SOD1 in the commonly studied G93A transgenic model were generated. Symptoms were monitored, CNS was examined histopathologically, spinal ventral neurons were counted by stereology and SOD1 aggregation was determined by a dot-blot filter assay.

Results: The mice developed an ALS-like syndrome and became terminally ill after around 370 days. The loss of spinal ventral neurons was similar to that in the G93A and other mutant SOD1 ALS models, and large amounts of aggregated SOD1 were found in spinal cords, but also in the brain.

Discussion: The mice that expressed human wild-type SOD1 recapitulated all the major pathological features found in mice expressing mutant SOD1s.

Conclusions: Wild-type human SOD1 has the ability to cause ALS in mice, supporting the hypothesis that the protein more generally could be involved in the disease in humans.

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P39 EARLY CHANGES IN THE SPINAL CORD GENE EXPRESSION IN SOD1 G93A AMYOTROPHIC LATERAL SCLEROSIS MOUSE MODEL

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Keywords: microarray, pre-symptomatic, molecular biology

Familial ALS is mainly linked to dominant mutations in the gene for Cu/Zn superoxide dismutase (SOD1). Toxic signs from non neuronal cells, mainly astrocytes and microglia, have been proposed to be involved in the rapid evolution of the disease. However, little is known about the signalling that triggers the onset of the disease.

In order to better understand the triggering of ALS onset, the RNA of lumbar spinal cords of transgenic mice at the pre-symptomatic ages of 40 and 80 days and age-paired wild type controls were extracted and the microarray analysis of the whole mouse genome was performed. Data were analyzed by means of GeneSpring GX software (Agilent) and DAVID Bioinformatics Resources 6.7 was used for enrichment analysis. Quantitative PCR was conducted in order to validate microarray data.

Statistical analysis has pointed to 1318 differentially expressed genes (451 up and 867 down regulated) at 40 days and 1314 differentially expressed genes (814 up and 500 down regulated) at 80 days old ALS mice. The main processes pointed by functional annotation from enrichment analysis for 40 days were MAPKinase signaling, axon guidance, neurogenesis, and synapse. At the age of 80 days, the main processes were more related to mitochondria (electron transport, respiratory chain and oxidative phosphorylation), transcription regulation, acetylation and ubiquitination. Real time PCR data from 6 assays Taqman and 6 SYBR primer pairs have confirmed the microarray results. The results indicate important processes occurring at the pre-symptomatic period of the disease and further analyses are required to elucidate the main mechanisms that trigger the onset of ALS disease. Support: FAPESP, CNPq.

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P40 BIOCHEMICAL AND GENETIC CHARACTERIZATION OF FUS

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Keywords: FUS, Drosophila, structure-function relationship

Background: The RNA-binding protein fused in sarcoma/translated in liposarcoma (FUS/TLS) has been implicated in familial amyotrophic lateral sclerosis (fALS). The structure and physiological function of FUS have yet to be better defined. The mechanisms by which the fALS mutations in FUS cause cellular dysfunction is also unclear.

Objectives and methods: We use X-ray crystallography and NMR approaches to determine the structure of FUS so that the physiological function of FUS can be better understood. The etiology of FUS mutation mediated fALS is to be elucidated using a combination of proteomic, biochemical and genetic approaches.

Results, discussion and conclusion: We have determined the structure of the RNA-binding domain (RRM) of FUS using NMR and its nucleic acid binding site. We also determined the crystal structure of the nuclear localization sequence (NLS) of FUS in complex with the nuclear importer. The in-depth structural understanding provides critical insights into the physiology function of FUS. In addition, the biochemical characterization of FUS has guided us to generate novel tools for genetic studies using Drosophila models to understand what the critical contribution factors to ALS disease are. The findings from the studies provide a comprehensive understanding of FUS function under physiological and pathological conditions.

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P41 MODELS OF FUS/TLS-MEDIATED ALS

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Keywords: FUS/TLS, transgenic mice, RNA-binding proteins

Background: More than 30 dominant mutations in the nucleic acid binding protein FUS/TLS (fused in sarcoma/translocated in liposarcoma) cause ~5% of familial ALS cases and are associated with neuronal and glial cytoplasmic inclusions containing FUS/TLS. Multiple roles of FUS/TLS have been proposed in both the nucleus and the cytoplasm, but its functional relevance to motor neuron health has not been specifically defined.

Objectives: To test whether mutant FUS/TLS expression in transgenic mice causes a motor phenotype or alters cellular pathways involved in stress responses important for motor neuron homeostasis.

Methods: We have established transgenic mice that express human wild type (WT) FUS/TLS or mutant FUS/TLS (H517Q, R521G, or R495X) in CNS neurons and astrocytes driven constitutively by the mouse prion protein (PrP) promoter.

Results: We detected > 5-fold overexpression of the R495X mutant FUS/TLS (expressed in cytoplasm > nucleus) in five independent transgenic lines that we bred successfully (average transgene copy number = 8.3). In contrast, for lines expressing human WT, H517Q, or R521G FUS/TLS (expressed in nucleus > > cytoplasm) that could breed, we obtained much more modest expression levels and only low copy numbers of the transgenes. However, copy numbers of founders expressing nuclear > > cytoplasmic FUS/TLS that subsequently failed to breed were 3-4 fold higher, on average, than those of founders that could breed (9.8 *vs.* 2.4 for WT and 7.7 *vs.* 2.6 for H517Q). ALS-related FUS/TLS mutations near the C-terminus caused a variable extent of cytoplasmic FUS/TLS accumulation in spinal cord anterior horn cells and other neurons. A relatively high burden of cytoplasmic R495X FUS/TLS was tolerated in neurons for > 1 year without causing toxicity. Cytoplasmic FUS/TLS variants accumulated focally in the perikaryon in a pattern resembling Nissl

bodies without the formation of well-defined inclusion bodies. Transgenic PrP-FUS^{mutant} mice exhibited normal nuclear TDP-43 staining. In a subset of mice, FUS/TLS mutant expression was associated with age-related motor deficits (decreased mobility, generalized tremor, or circling behaviors), but these abnormalities were not consistently observed.

Discussion: These results are consistent with an inhibitory effect of nuclear FUS/TLS overexpression on breeding success and a relative tolerance of the cytoplasmically expressed R495X truncation variant in neurons *in vivo*. Our results suggest that cytoplasmic accumulation of FUS/TLS variants does not primarily drive toxicity to motor neurons but that dysregulation of nuclear FUS/TLS expression has adverse consequences. Ongoing experiments will assess the sensitivity of these mice to increased mutant gene dosage, decreased expression of endogenous mouse FUS/TLS, and external stresses (e.g., oxidative stress, inflammation, disruption of proteostasis, or injury) to determine whether FUS/TLS mutants impair neuronal homeostatic mechanisms that could be relevant to ALS.

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P42 PHENOTYPIC CHARACTERIZATION OF B6.CG-TG(PRNP-TARDBP*A315T)95BALO/J MICE

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Keywords: TDP-43, phenotyping, mouse model

Background: Tar DNA binding protein 43 (TDP43) mutations have been implicated as a cause of some rare cases of familial amyotrophic lateral sclerosis (ALS) frontotemporal dementia (FTD). TDP43 pathology, largely manifested as mis-localization of the protein to the cytoplasm, has been observed in many cases of sporadic ALS and FTD. Rodent transgenic models are developed as tools to accelerate the understanding of the biology of human disease and to test therapeutics. Numerous transgenic mouse models designed to over-express human mutant and wild-type TDP43 have been developed. The first that was reported and made available to the research community was the B6.Cg-Tg(Prnp-TARDBP*A315T)95Balo line (Prp-TDP43A315T mice). Initial reports from this line indicated that Prp-TDP43A315T mice develop motor neuron disease.

Objectives: The objective of this work was to broadly observe a line of congenic transgenic mice derived from B6.Cg-Tg(Prnp-TARDBP*A315T)95Balo line.

Methods: The Prp-TDP43A315T mouse line was bred and maintained using both male and female mice as heterozygous transgene carriers mated with non-transgenic mice. Mice were observed daily applying a modified SHIRPA protocol with a focus on motor function. Immunohistochemistry (IHC) was applied to CNS analysis of human TDP43, glial fibrillary acid protein (GFAP), and F4/80, a marker of mouse macrophages. Immunofluorescent (IF) labeling of Cd68 positive cells and neuromuscular junctions (NMJs) of the peripheral nervous system and muscle in the Prp-TDP43A315T mice was studied. Finally, food/water consumption and gastric motility of Prp-TDP43A315T mice were monitored.

Results: Male Prp-TDP43A315T mice successfully mate with non-transgenic females only 50% as frequently as normal non-transgenic pairings. Lifespan of Prp-TDP43A315T mice covaries with gender and litter. Male Prp-TDP43A315T mice had a median life span of 101 days while females survived to 137 days. IHC of human TDP43 in spinal cord revealed intense nuclear staining and lighter cytoplasmic staining in neurons of the ventral and dorsal horns. IHC of GFAP and F4/80 in the spinal cord demonstrated astrocytosis and microgliosis. IF analysis of Cd68 positive signal in sciatic nerve indicated presence of activated macrophages in peripheral nerve tissue. IF analysis of neuromuscular junctions in the gastrocnemius of Prp-TDP43A315T mice revealed abnormal NMJ morphology. Application of modified SHIRPA to characterize motor function of the Prp-TDP43A315T mice revealed abnormal reflexes and gait changes. Gross anatomical study at end stage revealed gastrointestinal pathology that may be the cause of death for a significant number of Prp-TDP43A315T mice.

Discussion: This effort has helped to expand the understanding of the phenotype of the Prp-TDP43A315T mice generated and reported on by Wegorzewska *et al.* Understanding the timing associated with both behavioral and pathological changes in both genders of the Prp-TDP43A315T mice may provide insight into how and whether this transgenic model should be used for the study of neurodegeneration.

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P43 HO-1 INDUCTION IN MOTOR CORTEX AND INTESTINAL DYSFUNCTION IN TDP-43 A315T TRANSGENIC MICE

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Keywords: TDP-43 A315T transgenic mice, autophagy, antioxidant enzyme

Background: TDP-43 was identified as a pathological protein of ubiquitin-positive inclusions in frontotemporal lobar degeneration (FTLD) and ALS. Aggregates of ubiquitinated proteins were observed in layer 5 pyramidal neurons in frontal cortex of TDP-43 A315T transgenic mice. However, no cytoplasmic TDP-43 aggregates were found in TDP-43 A315T mice.

Objectives: To investigate whether autophagic alteration, glial activation or induction of antioxidant enzymes exists in the motor cortex of TDP43-A315T mice and to explore the lethal cause of the transgenic mice.

Methods: Transgenic human TDP-43 A315T mice were originally derived from the B6.Cg-Tg (Prnp-TARDBP* A315T) 95Balo/J line from The Jackson Laboratory. TDP-43 A315T male mice (n = 12) and non-transgenic littermates (n = 12) were used for clinical observation, Western blotting, immunohistochemical, and pathologic studies.

Results: TDP-43 A315T protein was relatively rich in the brain than in the spinal cord. Motor cortex was one of the regions that highly expressed TDP-43 transgene. In this study, we found stress-responsive HO-1 induction and no

autophagic alteration in motor cortex of TDP-43 A315T transgenic mice. Glial activation, especially astrocytic proliferation, occurred in cortical layer 5 and sub-meningeal region. Interestingly, we noticed that progressively thinned colon, swollen small intestine and reduced food intake, rather than severe muscle weakness, contributed to the death of TDP-43 A315T transgenic mice. Increased TDP-43 accumulation in the myenteric nerve plexus and increased thickness of muscular layer of colon were related to the intestinal dysfunction.

Discussion: The results presented in this study strongly suggest that oxidative stress and reactive astrocytosis were involved in the injury of cortical motor neurons in TDP-43 A315T transgenic mice. It was reported that reduction in myenteric neuron number in colon can cause impaired motility and decreased diameter. Therefore, TDP-43 accumulation in the myenteric nerve plexus and neurodegeneration of myenteric neurons in colon may result in symptoms similar to hypoganglionosis. As far as we know, this is the first report that TDP-43 A315T could cause lethal intestinal dysfunction, whose pathogenesis needs to be addressed in the future.

Conclusions: TDP-43 A315T mutant caused stress-responsive HO-1 induction in motor cortex and that TDP-43 accumulation caused-intestinal dysfunction may be the main cause of death in TDP-43 A315T transgenic mice.

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P44 A ZEBRAFISH MODEL OF TDP-43 RELATED AMYOTROPHIC LATERAL SCLEROSIS (ALS) REVEALS A NOVEL FEEDBACK LOOP MEDIATED BY ALTERNATIVE SPLICING OF TARDBPL BY TARDBP

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Keywords: TDP-43, zebrafish, autoregulation

Objective: To study the role of TDP-43 in a zebrafish model of amyotrophic lateral sclerosis.

Background: A major RNA processing function of TDP-43 is regulation of splicing. In mouse and drosophila models, TDP-43 has been shown to be important during early embryogenesis. TDP-43 has been observed to mis-localize from nucleus to cytoplasm of the surviving motor neurons in postmortem brain and spinal cord tissues. Thus raising the possibility that the loss of nuclear function of TDP-43 might be responsible in disease pathogenesis. Zebrafish is a robust vertebrate model, which we have used as a platform to study loss of function effects of TDP-43.

Methods: We identified tardbp and tardbpl as zebrafish orthologues of TARDBP. We have used Antisense Morpholino Oligonucleotides to transiently knock down tardbp and tardbpl. We generated a stable tardbp mutant by TILLING, Y220X, which results in a nonsense mediated decay of tardbp. We studied motor neurons, axonal out growth, swimming behavior, survival and neuromuscular junction architecture, immuno blotting, immunohistochemistry and qRT-PCR to characterize and confirm our findings.

Results: In a transient knockout of tardbp resulted in a phenotype similar to ALS. The stable homozygous tardbp Y220X mutant zebrafish shows significant reduction in weight and length at 6 months of age ($p < 0.0001$). On further examination of the Y220X mutant reveals a novel regulatory loop involving tardbpl, which rescues loss of tardbp phenotype. Inhibition of this novel regulatory loop results in a severe motor phenotype with shortened motor axons, a curly tail, locomotion defects, cardiovascular failure and death at around 10 days post fertilization.

Conclusion: In absence of tardbp, a novel splicing event is activated, resulting in generation of a novel full length transcript (*tardbpl-FL*) that compensates for the loss of tardbp. This finding provides a novel *in vivo* model of TDP-43 autoregulation. We also show that simultaneous elimination of both zebrafish *TARDBP* orthologues results in a severe motor phenotype with shortened motor axons, a curly tail dysmorphic appearance, locomotion defects and death at around 10 days post fertilization. This double knockout *tardbp* null zebrafish model provides a basis for analyzing pathological *TARDBP* mutations and could also be exploited in screens for neuroprotective agents.

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P45 LOSS OF TDP-43 RESULTS IN AGE-DEPENDENT PROGRESSIVE MOTOR IMPAIRMENT AND NEUROPATHOLOGICAL ALTERATIONS, MIMICKING MOTOR NEURON DISEASE

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Keywords: TDP-43, motor dysfunction, autophagy

Background: TDP-43, nuclear protein regulating RNA processing, redistributes to the cytoplasm and forms aggregates which is the histopathological hallmark of sporadic ALS in affected motor neurons, suggesting loss of TDP-43 function causes neurodegeneration. TDP-43 knockout mice result in embryonic lethal phenotypes, and systemic postnatal deletion of this molecule led to rapid death. Although TDP-43-depleted models of drosophila and zebrafish exhibit neurodevelopmental deficits in motor axons, there is no report on the selective TDP-43 depletion in post-mitotic mammalian neurons. Therefore, we generated motor neuron-specific TDP-43 knockout (TDP CKO) mice and investigated the loss of TDP-43 function in post-mitotic motor neurons.

Methods: We generated TDP-43 floxed mice and crossed with VAChT-Cre mice, in which Cre expression is mostly restricted in post-mitotic motor neurons. Neurological and behavial assessments and pathological analyses were performed.

Results: TDP CKO mice developed progressive weight loss and motor impairment about the age of 60 weeks, whereas

the survival rate of TDP CKO mice was not altered, compared with that of the control mice. Pathological analyses showed progressive atrophy in TDP-43-lacking spinal motor neurons, loss of large motor axons, grouped atrophy of the skeletal muscles, and denervation in the neuromuscular junctions of TDP CKO mice. Morphological analysis of the trigeminal motor, facial and hypoglossal nuclei in 100-week-old TDP CKO mice revealed that TDP-43-lacking motor neurons were significantly smaller than those with TDP-43 or those of the control mice, although the number of motor neurons in TDP CKO mice was not altered. Retrograde Fluorogold labeling was attenuated in TDP-43-lacking motor neurons of TDP CKO mice. In addition, ultra structural analyses revealed accumulations of autolysosomes and autophagosomes in motor neuronal cell bodies and axons of TDP CKO mice.

Discussion: In the present study, we clarified that TDP CKO mice, in which TDP-43 was specifically knocked-out in post-mitotic motor neurons, developed a progressive motor neuronal degeneration as seen in ALS, suggesting that TDP-43 is essential for the long term maintenance of post-mitotic motor neurons in mice. Given that ALS is an age-related neurodegenerative disease, our TDP CKO mice appear to be a model that recapitulates the age-dependent phenotypes of ALS.

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P46 MUTANT UBQLN2 TRANSGENIC MICE RECAPITULATE HUMAN BRAIN UBIQUILINOPATHY AND COGNITIVE DEFICITS

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Keywords: UBQLN2, transgenic, model

Background: Protein aggregates with immunoreactivity to ubiquitin are a common pathological hallmark of major neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the molecular mechanism of protein aggregation and its relationship with related neurodegeneration remain largely unknown. Mutations in *UBQLN2*, which encodes the ubiquitin-like protein ubiquilin2, have recently been shown to cause a subset of ALS and ALS with dementia. The distribution of ubiquilinopathy in the central nervous system of ALS and ALS/dementia cases is well correlated with motor and cognitive symptoms. Preliminary functional studies using *in vitro* models suggested that mutant ubiquilin2 impairs ubiquitin-mediated protein degradation. However, this mechanism remains yet to be tested *in vivo*, especially in mammalian models. Moreover, the pathophysiological basis of ubiquilinopathy-linked dementia remains to be elucidated.

Objectives: This study aims to develop and characterize transgenic mice overexpressing mutant UBQLN2, which is linked to ALS and dementia in human patients.

Methods: Transgenic mice were developed using human genomic DNA with encodes the mutant ubiquilin2 protein, P497H. Transgenic mice were characterized using behavioral, biochemical, pathological and neurophysiological methods.

Results: The mutant *UBQLN2* transgenic mice developed behavioral abnormalities and ubiquilinopathy in the central nervous system, especially the hippocampus, thus recapitulating some key clinical and pathological features of dementia observed in the human patients. We observed co-localization of ubiquilin2 and proteasome subunits in the inclusions, which were predominantly distributed in the dendritic spines. The mutant *UBQLN2* impaired ubiquitinated protein degradation and led to a conversion of long-term potentiation (LTP) to Long-term depression (LTD) in the transgenic mice.

Discussion: Our data provide robust *in vivo* evidence that links impaired protein degradation to protein aggregation, dendritic spinal pathology, neurophysiological defect and neurodegeneration, implying a molecular pathway for development of therapeutic approaches for neurodegenerative disorders. Moreover, the mutant UBQLN2 transgenic mice may provide a novel animal model not only for further studying memory deficit and other defects in neurodegenerative disorders, but also for testing rational therapeutic interventions.

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P47 IMPAIRED UBIQUITIN–PROTEASOME SYSTEM ACTIVITY IN TRANSGENIC MICE EXPRESSING ALS/DEMENTIA-LINKED MUTANT UBQLN2

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Keywords: FTD, dementia, UBQLN2, proteasome, protein aggregation

Background: The ubiquitin-proteasome system (UPS) may be responsible for the accumulation of potentially harmful ubiquitinated proteins in neurodegenerative disorders. In ALS, UPS dysfunction may be central to motor neuron death. Recently, we showed that mutations in UBQLN2, which encodes the ubiquitin-like protein ubiquilin 2, cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Ubiquilin 2 is a member of the ubiquilin family, which regulates the degradation of ubiquitinated proteins. Functional analysis showed that mutations in UBQLN2 lead to an impairment of protein degradation. Therefore, our findings linked abnormalities in ubiquilin 2 to defects in the protein degradation pathway, abnormal protein aggregation and neurodegeneration, indicating a common pathogenic mechanism.

Objectives: To further explore the pathogenic mechanism of UBQLN2-mediated ALS and ALS/dementia and the effect of mutant UBQLN2 on the UPS.

Methods: We first developed transgenic mice expressing an ALS/dementia-linked UBQLN2 mutation (P497H) using the human genomic DNA transgene. To functionally investigate the UPS in ALS motor neurons *in vivo*, we crossed the UBQLN2^{P497H} transgenic mice with transgenic mice expressing a fluorescently tagged UPS reporter substrate (Ub^{G76V}-GFP). In Ub^{G76V}-GFP/ UBQLN2^{P497H} double-transgenic mice, we stained brain sections and performed immunohistochemisrty using antibodies generated against

ubiquilin2 and ubiquitin. Western blotting was also performed to confirm our results.

Results: Prominent ubiquilin2-positive inclusions were observed in the hippocampus of Ub^{G76V}-GFP/ $UBQLN2^{P497H}$ double-transgenic mice. Confocal microscopy shows co-localization of ubiquilin 2 with ubiquitin and Ub^{G76V}-GFP in these hippocampal inclusions, consistent with UPS impairment and accumulation of Ub^{G76V}-GFP due to deficient reporter degradation. Accumulation of ubiquitinated proteins and Ub^{G76V}-GFP in these mice was also confirmed using Western blotting.

Discussion and conclusion: Our data suggest that UPS impairment occurs in hippocampal neurons in $UBQLN2^{P497H}$ transgenic mice and may be central to neurodegeneration observed in UBQLN2-linked ALS and ALS/dementia.

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P48 ABSENCE OF UCHL1 LEADS TO MOTOR NEURON CIRCUITRY DEFECTS, SPINAL MOTOR NEURON VULNERABILITY AND PROGRESSIVE CORTICOSPINAL MOTOR NEURON DEGENERATION

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Keywords: UCHL1, CSMN degeneration, motor circuitry health

Background: UCHL1 (ubiquitin C-terminal hydrolase-L1) is critically important for ubiquitin-proteosome system (UPS), whose importance for ALS is strongly implicated with the recent identification of *VCP*, *p62*, and *Ubiquilin2* mutations in ALS patients. However, the impact of improper UPS on motor neuron health and motor neuron circuitry are not known. As a member of the deubiquitinating enzymes (DUB), UCHL1 has both hydrolase and ligase activities and maintain free ubiquitin levels and is found in ubiquitinated protein-containing inclusions including Parkinson's disease (PD), Alzheimer's disease (AD), frontotemporal dementia (FTD), and ALS. This suggests that UCHL1 is intimately involved in the control of ubiquitin homeostasis in neurodegenerative diseases, where protein aggregates are a hallmark.

Objectives: This study aims to understand the possible function of UCHL1 in motor neuron circuitry health.

Methods: Using the transgenic $UCHL1^{nm3419}$ mice, which lack all UCHL1 function, we have analyzed motor neuron function at multiple levels: 1) behavioral tests to evaluate overall motor function (rotarod, Digigait, and grip test); 2) *in vivo* analysis of motor function; 3) immunocytochemical analysis of muscle, spinal cord and cerebral cortex in combination with retrograde labeling of CSMN approaches by Fluoro-Gold and retrograde transduction via AAV2-2. In addition, we investigated UCHL1 expression in the motor cortex of both familial and sporadic ALS patients' post-mortem brain samples.

Results: Our results demonstrate defects in motor function, muscle, and neuromuscular junctions together with spinal motor neuron vulnerability and progressive corticospinal motor neuron (CSMN) degeneration. CSMN degeneration was first evident with vacuolization of apical dendrites, spine loss and activation of ER-stress. Comparative analysis of human sporadic and familial ALS motor cortex revealed that UCHL1 immunoreactivity is increased only in the Betz cells of patients.

Discussion: Our findings based on detailed behavioral, cellular and molecular analyses using retrograde labeling, molecular marker expression, AAV-mediated gene delivery and novel *in vivo* electrophysiological recordings have revealed the importance of UCHL1 for motor neuron health both in ALS patients and in transgenic mice which lack all UCHL1 function. Increase in UCHL1 immunoreactivity, especially in the Betz cells of fALS and sALS patients, further implicates a broad involvement of UCHL1 and UPS for motor neuron health. In the absence of UCHL1, motor neuron circuitry progressively degenerates in the UCHL1-/- mice, with prominent and progressive CSMN degeneration, potentially due to ER stress.

Conclusions: We report that UCHL1 is essential for motor circuitry and is especially important for CSMN health. We now characterize a novel mouse model for motor neuron diseases with CSMN involvement.

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P49 THE IMPACT OF H63D HFE GENE VARIANT ON DISEASE PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: H63D HFE, SOD1 G93A/HFE+/H67D mice, SOD1 G93A mice

Background: The most common HFE gene variant, H63D HFE, is associated with 2.5-fold increased risk of developing amyotrophic lateral sclerosis (ALS). The H63D HFE is associated with iron accumulation, oxidative stress, abnormal glutamatergic secretion and prolonged endoplasmic reticulum stress, each of which is proposed as a contributing factor to ALS pathogenesis.

Objectives: To determine whether the presence of H63D HFE impacts disease onset and/or progression using a double transgenic mouse line that carries H63D HFE and SOD^{G93A} mutation.

Methods: We crossed an ALS mouse model (SOD^{G93A}) with H67D mice (homologous to H63D in human). The resulting double transgenic mice ($SOD^{G93A}/HFE^{+/H67D}$) are heterozygous for the H67D HFE and also carry the SOD^{G93A} mutation. 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

Motor neuron survival and microgliosis were determined by cresyl violet and Iba-1 immunohistochemcial staining with lumbar cord sections from presymptomatic (90-day), symptomatic (110-day) and end-stage mice. In all experiments, $SOD1^{G93A}/HFE^{+/H67D}$ mice were compared with $SOD1^{G93A}/HFE^{+/-}$ and $SOD1^{wt/wt}/HFE^{+/H67D}$ littermates.

Results: The survival of female $SOD^{G93A}/HFE^{+/H67D}$ mice was significantly reduced when compared with female SOD^{G93A} mice. Female $SOD^{G93A}/HFE^{+/H67D}$ mice also exhibited significantly shorter disease duration than SOD^{G93A} mice. The survival and disease duration of male $SOD^{G93A}/HFE+^{/H67D}$ was not different from male SOD^{G93A} mice. Age of onset in both males and females was not significantly different between $SOD^{G93A}/HFE^{+/H67D}$ and SOD^{G93A} mice. Compared to $SOD1^{wt/wt}/HFE^{+/H67D}$ mice, a significant loss of motor neurons and increased microgliosis were observed in $SOD1^{G93A}/HFE^{+/H67D}$ and $SOD1^{G93A}/HFE^{+/-}$ by 90-day and worsened with age. Motor neuron loss and microgliosis in $SOD1^{G93A}/HFE^{+/H67D}$ mice did not differ significantly from $SOD1^{G93A}/HFE^{+/+}$ mice at any observation age.

Discussion and conclusions: The H67D HFE (homologous to human H63D) reduced survival and disease duration in the *female* ALS mouse model; however, there is no effect on motor neuron survival or microglial activation. Additional evaluation of histopathology and biochemical pathways to determine the mechanisms by which H63D HFE contributes to accelerated disease in ALS is still in progress. Together with epidemiological data, the animal model data support that the H63D HFE is a contributing factor to ALS pathogenesis and its effect may be gender-dependent. Because as many as 30% of ALS patients carry the H63D variant, the animal model presented here is a significant tool for determining pathobiology of ALS and as a preclinical model for evaluating targeted treatment strategies which may be suitable for a subset of ALS patients with H63D HFE.

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P50 SELECTIVE INHIBITION OF HDAC6 RESTORES A DISTAL HEREDITARY MOTOR NEUROPATHY

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Keywords: distal hereditary motor neurophaty, mouse model, HDAC6

Background: Distal hereditary motor neuropathy (distal HMN) shows both clinical and genetic overlap with distal spinal muscular atrophy (distal SMA) and amyotrophic lateral sclerosis (ALS). The clinical signs include progressive and length-dependent muscle weakness and atrophy, reduced or absent deep tendon reflexes, foot and hand deformities. In more severe cases, affected humans become wheelchair-bound. Three out of 7 distal HMN-linked genes encode small heat-shock proteins (HSPB1, HSPB3 and HSPB8). The exact nature of the pathogenic mechanism underlying mutant HSPB1-induced distal HMN is unknown.

Objectives: We investigated the role of mutant HSPB1 in distal HMN. These insights could offer perspectives for the development of new therapeutic strategies.

Methods: We generated transgenic mice that selectively express human wild type or P182L-HSPB1 in postnatal neurons using the Thy1.2 expression cassette. We characterized these transgenic mice by means of behavioral tests, nerve conduction velocity studies and histological examination. We used these transgenic mice to identify a potential therapeutic target for distal HMN.

Results: P182L-HSPB1 mice display limb-clasping behaviour from 6 months on. Mutant HSPB1 mice show reduced rotarod performance aggravating in function of age. Muscle force is severely affected in hind limbs while forepaw muscle force decreased only later in disease. P182L-HSPB1 mice also demonstrate steppage gait. Sensory axons of P182L-HSPB1 are unaffected. Nerve conduction studies confirm the presence of motor axonopathy in P182L-HSPB1. Histochemical analysis of muscle, neuromuscular junctions and peripheral nerves show signs of peripheral neuropathy. Acetylated tubulin abundance is decreased in peripheral nerves of symptomatic P182L-HSPB1 mice. Three weeks of daily treatment with a selective histone deacetylase 6 (HDAC6) inhibitor, tubastatin A, restores the levels of acetylated tubulin in peripheral nerves of symptomatic P182L-HSPB1 mice. This treatment strategy results in a rescue of rotarod performance and an increase in number of functional axons.

Discussion: The selective expression of P182L-HSPB1 in neurons gives rise to a distal HMN phenotype in mice. Thus, mutant HSPB1 cell-autonomously leads to peripheral neuropathy. Mutant HSPB1 induces a reduction of acetylated tubulin abundance in peripheral nerve of symptomatic mice. Selective HDAC6 inhibition restores acetylated tubulin levels and rescues the distal HMN phenotype both behaviourally and functionally.

Conclusions: This work describes a new mouse model for distal HMN. This mouse model accurately recapitulates all key symptoms of the human condition. Moreover, selective inhibition of HDAC6 restores acetylated tubulin abundance in peripheral nerve and rescues the distal HMN phenotype both behaviourally and functionally. HDAC6 could be an attractive therapeutic target for other motor neuron disorders within the spectrum of neurodegenerative disorders.

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P51 CANINE DEGENERATIVE MYELOPATHY, AN ALS MODEL, IS WIDESPREAD IN THE PET POPULATION

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Keywords: degenerative myelopathy, SOD1, dog

Background: Canine degenerative myelopathy (DM) is a spontaneously occurring adult-onset neurodegenerative disease

The initial signs typically include asymmetric general proprioceptive ataxia and spastic paresis in the pelvic limbs together with spinal reflexes that suggest upper motor neuron loss. The disease progression is relentless and dogs maintained beyond a year after disease onset become nonambulatory paraparetic or paraplegic. If euthanasia is further delayed, the dogs develop paralysis in the pelvic limbs associated with lower motor neuron dysfunction. Because a variety of common acquired compressive spinal cord diseases can mimic the early stages of DM by compromising the upper motor neuron pathways, a definitive diagnosis of DM can only be accomplished *postmortem* by the histopathologic observation of axonal and myelin degeneration and astrogliosis in spinal cord funiculi. In 2008 we reported an association between DM and homozygosity for a c.118G>A missense mutation in the canine ortholog of *SOD1* in five dog breeds, chosen for sample availability. Since then we have genotyped over 25,000 dogs at *SOD1: c.118G.A* and over 1,500 dogs at *SOD1: c.52G.T*, a second DM-associated *SOD1* missense mutation. In addition, we have examined 231 canine spinal cords for the presence of histopathologic lesions consistent with DM.

Objectives: The objectives were to determine the distribution of the *SOD1: c.118A* and *SOD1: c.52T* alleles in the canine pet population and to determine how the presence of these alleles correlates with the development of clinical DM.

Methods: Routine molecular-genetic and histopathologic techniques were used in this study.

Results: A total of 26,898 individual dogs representing 217 different breeds were genotyped at *SOD1: c.118G.A*. Approximately half of these dogs (n = 13,453) were homozygous for the wild-type G allele; whereas, 7,071 dogs (26.3%) were A/G heterozygotes and 6,374 (23.7%) were homozygous for the DM-associated A allele. The allele frequency of A allele for all genotyped dogs was 36.8%. The A allele was detected in at least one representative from 115 breeds which was 53% of the examined breeds. A total of 1,577 dogs were also genotyped at the *SOD1: c.52A.T* mutation. Only the wild-type A allele was detected in 693 dogs representing 59 breeds; however, among the 884 genotyped Bernese Mountain Dogs, 53 were A/T heterozygotes and two homozygous for the *SOD1: c.52T* allele. Thus, the *SOD1: c.52T* allele frequency among the 884 genotyped Bernese Mountain Dogs was 3.2%. There were correlations between clinical DM, homozygosity at *SOD1: c.118A*, homozygosity at *SOD1: c.52T*, and compound heterozygosity at *SOD1: c.118A/G* and *SOD1: c.52A/T*.

Discussion and conclusion: The widespread distribution of the *SOD1: c.118A* allele in the pet dog population indicates that there is an available resource of privately owned dogs that could participate in clinical trials of interventions that could potentially help both ALS and DM patients.

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P52 MOTOR UNIT PATHOLOGY IN CANINE DEGENERATIVE MYELOPATHY

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Keywords: morphometry, dog model, motor unit

Background: Canine Degenerative Myelopathy (DM) is a late onset, heritable neurodegenerative disease that occurs in several breeds. Initial clinical signs of DM include spastic paraparesis in the pelvic limbs that progresses to flaccid tetraplegia and brain stem involvement .We previously identified a missense mutation in the canine superoxide dismutase 1 (*SOD1*) gene in DM-affected dogs. DM pathology and disease progression is similar to upper motor neuron onset ALS. The pathologic hallmarks of ALS include muscle degeneration, axonal loss, extensive astrogliosis, and neuronal cell body death in upper and lower motor neurons. Prominent pathologic features of DM include axonal loss, and astrogliosis, most severe in the dorsal portion of the lateral funiculus and in the dorsal columns of the middle to lower thoracic spinal cord (SC). These findings suggest that DM may be similar to at least some forms of human ALS.

Objectives: For many years, pathology of DM was considered to only involve the spinal cord tracts; however, recent histopathologic and electrophysiologic studies have confirmed involvement of lower motor neurons (LMNs). Thus, in an effort to further assess DM as a potential ALS research model, a study was undertaken to further explore possible LMN involvement in DM disease progression. We hypothesize that there will be a loss of motor neurons (MNs), loss of myelinated axons, and/or neurogenic atrophy in muscles involving thoracic motor units of DM-affected Boxers and Pembroke Welsh Corgis (PWC).

Methods: Anti-choline acetyl transferase was used to label MNs in Rexed-laminae IX of thoracic SC segment 7 of control and DM-affected dogs. Labeled MNs were counted and areas measured using ImageJ software™. Sections of fixed thoracic SC segment 8 (T8) motor roots were stained with P-Phenylene diamine. Computer-assisted and manual techniques were used to count and obtain morphometric measurements of myelinated axons. H&E stained cross sections of intercostal muscle were evaluated by light microscopy for evidence of denervation atrophy.

Results: No losses of MNs were detected in the thoracic cord of DM-affected dogs, and T8 root axon counts and size distribution analyses indicate there is no loss of somatic motor axons. Marked variability of myofiber size and shape was observed only in end stage PWCs, suggestive of denervation atrophy.

Discussion and conclusions: DM does not appear to involve loss of MNs in the thoracic SC or proximal degeneration of the axons of these neurons. However in PWCs with later disease stage, neurogenic muscle atrophy was observed. Lack of MN and motor axon loss, with evidence of neurogenic muscle atrophy in end-stage suggests that DM may reflect distal axonal degeneration and/or impairment in neuromuscular transmission. Further research of motor units from other spinal cord levels will be necessary to elucidate the mechanisms underlying neuromuscular pathology in DM.

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THEME 3 *IN VITRO* EXPERIMENTAL MODELS

P53 DEVELOPMENT OF AN *IN VITRO* MODEL OF HUMAN MOTOR NEURONS FROM HUMAN FOETAL SPINAL CORD IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: human primary motor neuron culture, spinal cord

Background: Human primary motor neuron (MN) isolation provides a critical tool to study neuropsychological properties and the effects of molecules of clinical relevance on isolated neurons. We developed a technique using a combination of centrifugation and density gradient separation process from foetal spinal cord.

Methods: We have isolated human motor neurons from spinal cord of human foetal brain (16–20 week of gestation). At day 3 *in vitro* (DIV), 1-β-D arabino furanosylcytosine (Ara-C) was used to reduce non-neuronal cell growth. The purity of motor neuron culture were then assessed using immunocytochemistry with $p75^{NTR}$, SMI-32 mAbs and cell counting. Following dissociation of spinal cords and separation by gradient centrifugation, cells from the top layer located at the medium were analyzed 7 days after plating by immunocytochemistry using a labeling with SMI-32 antibody and a staining of the nuclei with DAPI. This high purity was confirmed by flow cytometry quantification of motor neuron purity following labeling with anti-$p75^{NTR}$ or without primary antibody as a control for the background level.

Results: We have obtained an almost pure (SMI-32+, $p75^{NTR}$) primary culture of human motor neurons. The percentage of SMI-32-positive and $p75^{NTR}$ -positive motor neurons relative to the number of cell nuclei was 86.17 ± 2.78 and 84.74 ± 3.26 when Ara-C was treated.

Conclusions: We have successfully optimized a new method to obtain highly purified primary culture human motor neuron. This tool will be very important to study neurotoxicity and neuroprotection to assess therapeutic strategies for motor neuron diseases.

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P54 A NOVEL METHOD FOR CREATING PLURIPOTENT STEM CELL LINES FROM SOMATIC TAIL TIP CELLS FROM TDP-43 TRANSGENIC MICE

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Keywords: iPS, somatic tail tip, TDP-43

Background: Reprogramming adult mammalian cells to generate pluripotent stem cells has been a seminal event in biology. The ectopic expression of the transcription factors Oct4, Sox2, c-Myc and Klf4 using viral vectors, nucleic acid transfection, or chemical induction have been a necessary step in the initiation of reprogramming for the generation of induced pluripotent stem (iPS) cells. Unfortunately, the most efficient method using genome integrating viral vectors, such as lentivirus, carries a significant risk of generating transformed cells. This problem has limited the utility of viral vector reprogrammed iPS lines in transgenic studies.

Objectives: Establishment of murine pluripotent stem cell lines from quiescent stem cells in the tail tip of a TDP-43 transgenic mouse line without reprogramming using viral vectors encoding the transcription factors Oct4, Sox2, c-Myc and Klf4.

Description: The method is based on the isolation and purification of an endogenous stem cell population present in the most distal tip of the adult mouse tail. Using tail tip biopsy from non-transgenic C57Bl6J mice and a transgenic TDP-43 mouse Balo line 1 mm lengths of tail tips were harvested, the superficial dermis peeled away and the remaining tissue cut into 1-mm pieces using a scalpel and cultured. Following cell outgrowth the tissue pieces are removed using sterile technique. The remaining cells, adhering to the tissue culture well, are allowed to proliferate until confluent. At this time the cells are passaged and expanded. Prior to molecular analysis of pluripotency the cells are plated onto a glass slide chamber coated with poly-lysine and allowed to form embryoid bodies.

Results: The cell lines shown no signs of senescence and have been passaged repeatedly. Using standard antibody stem cell marker panel the cell lines were positive for Oct3/4, Sox2, Nanog, and SSEA1 by immunohistochemistry and positive by western blot for Oct3/4, Sox2, and Nanog. This was also confirmed by qPCR for pluripotency markers. These cells have proven useful in the establishment of a cell-based assay of TDP-43 localization in response to cell stress.

Conclusions: Using organ cultures of the murine tail tip we have isolated stem cell lines positive for known markers of pluripotency. This method has the advantage of simplicity and avoidance of viral reprogramming strategies.

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P55 MUTANT INDUCED PLURIPOTENT STEM CELL LINES RECAPITULATE ASPECTS OF TDP-43 PROTEINOPATHIES AND REVEAL CELL-SPECIFIC VULNERABILITY

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Keywords: iPSCs, TDP-43, disease modelling

Background: Transactive response DNA-binding (TDP-43) protein is the dominant disease protein in amyotrophic lateral sclerosis (ALS) and a subgroup of frontotemporal lobar degeneration (FTLD-TDP). Mutations in the gene encoding TDP-43 (*TARDBP)* linked to familial ALS are shown to be neurotoxic *in vitro* and *in vivo*, mechanistically implicating pathological TDP-43 in disease processes. The molecular basis underlying selective vulnerability and progressive degeneration of motor neurons in motor neuron disease (MND) is not clear, although contribution of non-cell autonomous mechanisms to pathology is increasingly recognized. The discovery of ubiquitinated TDP-43 cytoplasmic inclusions as a unifying pathology across ALS and FTLD-TDP cases raises the need to develop experimental platforms that can investigate mutant and wild-type TDP-43 protein turnover in various functional cell types including spinal motor neurons (MNs), astrocytes, corticospinal MNs and superficial and deep-layer cortical neurons, all derived from the same genetic background.

Objectives: In order to establish a platform for *in vitro* disease modeling we have optimized differentiation protocols to generate spinal motor neurons and astrocytes from pluripotent stem cells and generated induced-pluripotent (iPS) cell-lines from TDP43 M337V mutation carrying and control individuals.

Methods: iPS cell-lines from adult human fibroblasts were generated by viral transduction of Oct4, Sox2, Klf4 and Myc and validated by standard criteria. Generation of functional neurons and glia was assessed by IHC, PCR, electrophysiology and resulting populations were further characterized by quantitative biochemistry, immunofluorescence densitometry and longitudinal microscopy for survival analysis.

Results: Neuronal cultures carrying the M337V TDP-43 mutation display increase in TDP-43 protein levels, fragmentation, accumulation in detergent-resistant fraction and cytoplasmic mislocalisation in parallel with the histological and biochemical signature of human TDP-43 pathology. Moreover, the TDP-43 M337V mutation confers a cell autonomous vulnerability to human MNs as shown by longitudinal survival analysis and increased vulnerability to antagonism of the PI3K pathway. We further develop these observations using astrocytes derived from M337V TDP-43 and control iPSCs.

Discussion: Our findings demonstrate the utility of pluripotent stem cell-derived neurons and glia in modeling molecular pathogenesis of neurodegenerative disorders at physiological protein expression levels.

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P56 KINASE CONTROL OF TDP-43 ACCUMULATION: POTENTIAL THERAPEUTIC TARGET FOR TREATMENT OF TDP-43 PROTEINOPATHIES

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Keywords: TDP-43, stress granules, kinase

Background: TDP-43 mis-localization and accumulation is central to the disease process in TDP-43 proteinopathies including motor neuron disease and frontotemporal dementia. However, the mechanisms leading to TDP-43 accumulation are not well understood. TDP-43 is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family of DNA and RNA binding proteins. It is known that movement of hnRNPs within cells is controlled by several kinases and therefore possible that these kinases could also control TDP-43 accumulation.

Objectives: In this study, we investigated whether kinases are important to controlling movement and accumulation of TDP-43.

Methods: SH-SY5Y, HeLa or primary cortical astrocyte cultures were treated with inducers of stress including paraquat or arsenite. In addition, SH-SY5Y cells were transfected with GFP-tagged C-terminal TDP-43 (219–414). We investigated the effect of various kinases on cytosolic accumulation of TDP-43 using a range of kinase inhibitors in conjunction with immunofluorescence analysis of TDP-43 and additional stress granule proteins. The localization of activated kinases was also determined by immunofluorescence. Immunoprecipitation was used to determine TDP-43 protein binding partners.

Results: We have found that c-Jun N-terminal kinase (JNK), cyclin-dependent kinase 2 (CDK2) and glycogen synthase kinase 3 (GSK3) have important roles in controlling TDP-43 accumulation in a variety of cellular models. Inhibitors of these kinases blocked TDP-43 accumulation with limited effect other ubiquitous stress granule markers such as human antigen R (HuR). Each of the target kinases also co-localized with TDP-43 in cytoplasmic stress granules. While JNK and GSK3 were important for initial TDP-43 accumulation, CDK2 appeared to have a key role in maintaining TDP-43 in aggregates. While the mechanism of action for each kinase is still under investigation, our preliminary findings strongly suggest that kinases may control TDP-43 movement and accumulation by phosphorylation of binding partners such as hnRNP K.

Discussion: Our studies have shown an important role for kinase activity in controlling TDP-43 movement and accumulation, central facets of TDP-43 proteinopathies.

Conclusions: Kinase control of TDP-43 localization may provide a valuable target to inhibit abnormal TDP-43 metabolism. This may be achievable through use of kinase inhibitors currently in clinical trials for other diseases.

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P57 QUANTITATIVE MASS SPECTROMETRY BASED PROTEOMICS A NEW PERSPECTIVE ON NEURODEGENERATION

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Keywords: quantitative, proteomics, interactions

Background: Dominant gain of functions is associated with a variety of mutant genes in ALS. However, for the majority their mode of action in ALS pathology is unknown. To reveal common features of ALS linked mutant proteins and uncover the reason for the cellular specificity are the primary goals of our proteomics approach.

Objectives: We investigate pathogenic mechanisms in ALS using cell culture models and state of the art mass spectrometry (MS) based proteomics. Advanced equipment in house developed algorithms are used to identify and quantify almost all cellular proteins within reasonable time. We set up a protein-protein interaction screen using wild-type and mutant epitope tagged proteins of ALS associated genes as baits in cell lines such as the motor neuron like NSC-34 cells. The identification of novel interaction partners or common patterns in the interactome of ALS linked proteins will broaden our understanding of ALS pathology.

Preliminary results: We labeled NSC-34 cells with heavy arginine and lysine using Stable Isotope Labeling by Amino acids in Cell culture (SILAC). Cell culture conditions were optimized to avoid metabolic conversion while ensuring high incorporation (>97%) of heavy amino acids. Beside the SILAC approach, label free, XIC based algorithms are utilized to quantify proteins in huge sets of different tissue samples.

Discussion and outlook: Establishing the heavy labeled NSC-34 cell line provides a unique tool for high resolution proteomics. Ectopic expression of ALS associated genes in cell lines can be used to investigate cell-type specific protein-protein interactions and to elucidate changes within the proteome of the cells.

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P58 PROTEOMIC ANALYSIS OF PROTEINS INTERACTING WITH THE RNA BINDING PROTEIN FUS

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Keywords: FUS, proteomics, protein interaction

Background: Mutations in the RNA binding protein fused in sarcoma (FUS) have been linked to familial ALS recently. However, the role of FUS in the ALS pathogenesis is largely unknown. Identification of interacting proteins of wild-type FUS and the disease-causing mutants is a prerequisite for the understanding of the functions of FUS. In this study, mass spectrometry-based proteomic method combined with GST pull-down technique is used to determine the protein-protein interaction of FUS.

Methods: N2A cells were transfected with GST-tagged wild-type and mutant FUS constructs. Cell lysates were prepared in RIPA buffer and GST pull-down was carried out using glutathione-Sepharose 4B beads. Alternatively, the cell lysate was treated with RNAse prior to the GST pull-down, which would allow the isolation of RNA-independent FUS-interacting proteins. The glutathione beads were collected, washed, and boiled in 2xSDS sample loading buffer. Proteins released from the beads were resolved by SDS-PAGE and stained with SyproRuby. Protein bands were sliced and subjected to in-gel trypsin digestion. LC-MS/MS analysis was performed utilizing an LTQ-Orbitrap mass spectrometer, equipped with a nanospray ion source and coupled with an Eksigent Nanoflex cHiPLC^{TM} system. A gradient of mobile phases (flow rate 300nL/min) containing 0.1% (v/v) formic acid in water and acetonitrile was applied. A data dependent mass analysis method with dynamic exclusion was utilized. The mass data were submitted to a local mascot server for an MS/MS protein identification search. Maximum error tolerance is 10 ppm for MS and 0.8 Da for MS/MS. Proteins with two or more high confidence peptides were considered unambiguous identifications without manual inspection. Proteins identified with one high confidence peptide were manually inspected and confirmed.

Result and discussion: A cohort of proteins interacting with both wild-type FUS and disease-causing mutants were indentified utilizing the mass spectrometry-based proteomic method. The proteins of interest were subjected to immunoprecipitation and Western blot for independent validation. By comparing the identified interacting proteins with and without prior RNAse treatment, the RNA-dependence of the FUS-interacting proteins was also determined. The functional studies of the interacting proteins are ongoing and the results will be presented.

Conclusion: We have used mass spectrometry-based proteomic approaches to identify proteins interacting with FUS and determine the RNA-dependence of the interaction. Results from this study provide critical insights into the FUS function in the physiological and pathological conditions.

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P59 A NEW MOLECULAR MECHANISM DESCRIBING THE FORMATION OF DISULFIDE CROSS-LINKED SOD1 IN THE FAMILIAL FORM OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1, disulfide bond, protein aggregation

Background: Cu,Zn-superoxide dismutase (SOD1) is a protein in which the folding/misfolding is regulated by the thiol-disulfide status as well as binding of copper and zinc ions. Indeed, the disulfide-reduced state of demetallated (apo) SOD1 easily forms amyloid-like fibrillar aggregates *in vitro*

which would describe the formation of Thioflavin S-positive inclusions in ALS-model mice. Given highly reducing environment of the cytosol, reduction of the intramolecular disulfide bond in mutant SOD1 appears to be a key event for triggering SOD1 aggregation inside the cell. While significant amounts of disulfide-reduced SOD1 have been reported to constitute the insoluble aggregates in ALS model mice, non-native inter-molecular disulfide bonds have also been found to cross-link the SOD1 oligomers. Therefore, abnormal redox chemistry of Cys residues in SOD1 appears to operate in misfolding and aggregation of mutant SOD1, but it remains unknown how the thiol-disulfide status of SOD1 is changing during the pathological process of SOD1 aggregate formation.

Objectives: SOD1 has four Cys residues of total, among which Cys 57 and 146 forms an intramolecular disulfide bond. To reveal a molecular mechanism describing the formation of disulfide-linked SOD1 aggregates, we have examined *in vitro* aggregation of SOD1 with and without the disulfide bond and tested if any changes occur in the disulfide linkage among the four Cys residues.

Methods and results: Human SOD1 proteins overexpressed in *E.coli* were purified in the apo form with a disulfide bond between Cys 57 and 146 (apo-SOD1$^{S\text{-}S}$), and its aggregation was monitored by increases in the solution turbidity. As reported previously, no aggregation of apo-SOD1$^{S\text{-}S}$ was confirmed; however, addition of a chaotropic reagent, guanidine hydrochloride (Gdn), was found to trigger aggregation. Furthermore, electrophoretic analysis on such Gdn-triggered apo-SOD1$^{S\text{-}S}$ aggregates showed the formation of disulfide-linked oligomers. To identify which of the four Cys residues form the disulfide linkages in the SOD1 oligomers, we have further performed a peptide-mapping analysis by mass spectrometry; as a result, a disulfide bond in oligomeric SOD1 was no longer observed between Cys 57 and 146 but was found to be scrambled with the other two Cys residues, Cys 6 and 111.

Discussion and conclusions: Here, we have proposed for the first time the abnormal scrambling of a disulfide bond in SOD1 proteins. Structural destabilization of apo-SOD1$^{S\text{-}S}$ triggers the nucleophilic attack of Cys 6/111 to the disulfide bond between Cys 57 and 146. Such disulfide isomerization first occurs within an SOD1 molecule and then appears to proceed between the molecules. The disulfide reduction is thus not always required for SOD1 misfolding; rather, the disulfide scrambling by intra- and inter-molecular isomerization also constitutes a pathologically-relevant pathway for the aggregation of mutant SOD1 proteins.

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P60 POST-TRANSCRIPTIONAL REGULATION OF SOD1 MRNA LEVELS THROUGH THE 3'UTR BINDING OF THE ELAV PROTEINS

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Keywords: SOD1 mRNA, ELAV proteins, motor cortex

Background: Increased levels of SOD1 mRNA have been observed in nervous areas affected by ALS disease and in PBMCs from sporadic ALS (SALS) patients compared to controls (1). Consequently, the understanding of the mechanisms by which *SOD1* gene expression is modulated may shed new light on SOD1 involvement in ALS. In particular, it is well known that post-transcriptional mechanisms represent an additional regulatory mechanism for gene expression. To this regard, some adenine/uridine-rich elements in the 3'UTR of SOD1 mRNA were identified (2). These elements represent the docking sites for many RNA-binding proteins among which a relevant place is taken by ELAVs that act mainly as positive regulators of gene expression.

Objectives: We aimed to investigate, using an *in vitro* cellular model (SH-SY5Y), whether SOD1 mRNA represents a target of ELAVs, and whether the binding between these proteins and SOD1 mRNA, and consequently SOD1 protein expression, is favored by oxidative stress, a condition observed in ALS. Furthermore, we wanted to investigate possible changes of ELAVs expression in the motor cortex from SALS patients.

Methods and results: RNA Electrophoretic Mobility Shift Assay (REMSA) was used to test the association between purified ELAV/HuR protein and ARE elements in the 3'UTR of SOD1 mRNA. SH-SY5Y cells were treated with 1mM H_2O_2 and RNA-immunoprecipitation (RIP) assay was performed to corroborate this molecular interaction. SOD1 protein expression and ELAVs localization in SH-SY5Y cells was evaluated by Western Blog. Immunohistochemistry analysis (IHC) was performed on motor cortex tissues from SALS patients and controls for both ELAVs and SOD1. By performing REMSA and RIP, we proved the molecular interaction between ELAVs and SOD1 mRNA observing that the cell treatment with H_2O_2 induced a significant increase of the binding. To further investigate the effects of the oxidative stress on ELAVs activation, we evaluated their subcellular localization in H_2O_2-treated cells and we found a significant increase in the cytoplasm. We then studied ELAVs expression and intracellular localization by performing IHC in the motor cortical tissues from SALS patients and controls. While in the control tissues ELAVs appeared mainly in the nucleus, in the sections from SALS subjects they were localized also in the cytoplasm.

Discussion and conclusion: This data suggest that the ELAVs may be more available and massively enrolled in the positive regulation of target mRNAs, such as SOD1. In support of this hypothesis, it is worth of note that both SOD1 mRNA and protein levels were increased in the motor cortex from SALS patients in comparison to controls. We demonstrated that ELAVs-mediated post-transcriptional events contribute to determine the levels of SOD1 mRNA and protein. These findings shed new light on *SOD1* gene expression regulation, disclosing new mechanisms involved in ALS.

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P61 DYSREGULATION OF AMP-ACTIVATED PROTEIN KINASE SIGNALLING IN MODELS OF ALS

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Keywords: AMP kinase, energy, homeostasis

Background: Increasing evidence implicates a role for abnormal energy homeostasis in ALS patients and mouse models. However, the role of energetic defects in motor neuron degeneration and loss remains unclear. AMP activated protein kinase (AMPK) is a key metabolic and stress sensor activated under conditions of energy depletion and responds early to cellular stress. Therefore, we hypothesise that energetic disturbances regulated by AMPK may contribute to motor neuron degeneration in ALS.

Objectives: To examine AMPK pathway activation and signalling in the motor neuron cell line NSC-34 and mouse models expressing ALS-linked mutant proteins; SOD1, TDP-43 or FUS. Also, to determine whether cell lines expressing mutant SOD1, TDP-43 or FUS are more susceptible to agents that disrupt energy homeostasis.

Methods: NSC-34 cells stably expressing normal or mutant SOD1, TDP-43 or FUS were analysed for AMPK signalling pathway markers by Western blotting and immunocytochemistry. Energetic markers were also examined in spinal cords of transgenic $SOD1^{G93A}$ and $TDP\text{-}43^{A315T}$ mice at various disease stages. Lastly, stable cell lines were treated with toxins targeting mitochondrial and energetic pathways to determine survival.

Results: We observed that AMPK phosphorylation was significantly increased in NSC-34 cells expressing mutant SOD1, as well as in spinal cord lysates from transgenic $SOD1^{G93A}$ mice as early as 30 days old. AMPK activation was not observed in non-neuronal HEK293 cells expressing SOD1 mutants, suggesting that it is neuronal-selective. In contrast, AMPK activation was significantly impaired in NSC-34 cells expressing mutant TDP-43 compared to wild-type protein and was correlated with increased nuclear depletion and cytoplasmic redistribution of mutant TDP-43. We also demonstrated that NSC-34 cells were highly susceptible to energy and mitochondrial disruption which was enhanced by expression of SOD1 and TDP-43 mutations.

Discussion: Our data suggest that AMPK activity is abnormal in cell and mouse models expressing SOD1 and TDP-43 mutations. Our finding that AMPK activity is stimulated in pre-symptomatic $SOD1^{G93A}$ mice preceding motor neuron degeneration indicates that AMPK might play an early role in ALS pathogenesis. We will continue to define the downstream targets of AMPK activation and link aberrant energy metabolism to motor neuron degeneration.

Conclusions: Our data implicate an early contribution of aberrant neuronal AMPK signalling in ALS models that is common to various ALS-linked misfolded proteins.

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P62 AN INFLAMMATORY RESPONSE IN PERIPHERAL NERVE AND CORRESPONDING SPINAL CORD IN WOBBLER MICE

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Keywords: wobbler mice, neuroinflammation, 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. Previous reports suggest that when activated, microgria can produce and release pro- and anti-inflammatory molecules, and may exert either a toxic or protective effect on neurons depending on the physiologic conditions.

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.

Methods: Cervical cord (C4)-phrenic nerve-diaphragm unit from 20 (early symptomatic stage), 40 (rapid progressive stage), 60 (chronic stage) days 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, BDNF, GDNF, IL-4, IL-1β and NOX2) and immunohistochemistry. Denervation was evaluated in diaphragm using quantitative RT-PCR for the temporal changes in the mRNA levels of AChRγ(fetal) and ε(adult) subunits. Data were analyzed using two-tailed Student's t test and group means were plotted ± SEM; $p < 0.05$ was considered statistically significant. Differences between groups were analyzed using a two-way ANOVA.

Results: Compared with WT control mice, both AChRγ subunit in diaphragm and CD68 (marker of moncytes/macrophages (microglia)) in cervical cord were elevated at 20 days. In addition, protective and anti-inflammatory factors, including BDNF, GDNF, IL-4 were increased in the cervical region at 20 days. However, the neurotoxic markers IL-1β, NOX2 were increased at 40 days.

Discussion: These results suggest that in cervical region of wobbler mice activate microglia produce and release protective and anti-inflammatory factors at the early symptomatic stage, and neurotoxic factors at rapid progressive stage.

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P63 TNF-ALPHA RECEPTOR TYPE 2 IS IMPLICATED IN MOTOR NEURON LOSS OCCURRING IN A CELLULAR MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TNFR2, TNFalpha, in vitro models

Background: Many studies over the years have demonstrated altered homeostasis of TNFa system in human patients and experimental models of ALS. Nevertheless, the actual contribution of TNFa in the genesis and progression of the disease is still controversial. TNFa exerts its activity through membrane receptors TNFR1 and TNFR2 that may have opposite effect in the neurodegenerative process. We and other groups found higher levels of TNFa and its receptors in the spinal cord of pre-symptomatic SOD1-G93A mice and in the blood of ALS cases. Moreover, thalidomide protected motoneurons in transgenic mice although the absence from the birth of TNFa in mutant SOD1 mice did not ameliorate the course of disease, raising doubts about its role in the disease development.

Objective: Therefore, we wished to further investigate the role of TNFa and its receptors TNFR1 and TNFR2 in the selective degeneration of motoneuron occurring in ALS using both *in vitro* and *in vivo* approaches.

Methods: We used an *in vitro* model consisting of astrocytes/spinal neurons cocultures expressing or not SOD1-G93A and transgenic SOD1-G93A mice also knock-out for TNFR1, TNFR2 or both. The effects of drug treatments and genetic manipulation were evaluated *in vitro* assessing large motorneuron viability and *in vivo* monitoring disease progression and survival of the mice.

Results: We observed a selective death of motoneurons in SOD1-G93A cocultures after 6 days that required the presence of astrocytes. The loss of motoneurons, which showed higher expression of intracellular and membrane-bound TNFa, was prevented by thalidomide and by the use of anti-TNFa antibody in coculture medium. Levels of TNFa in transgenic coculture medium were unchanged. TNFR2 was overexpressed in transgenic cocultures and in astrocytes in particular. Antibodies against TNFR2 and the use of astrocytes or neurons knock-out for the receptor rescued SOD1-G93A motoneurons. IgG fused to extracellular region of murine TNFR2 (solTNFR2) was ineffective in protecting motorneuron but caused the death of these cells in isolated neuronal cultures, in the absence of the astrocytes. Intracerebroventricular administration of solTNFR2 did not ameliorate symptoms and life span of SOD1-G93A mice. Furthermore, survival and disease progression of transgenic SOD1-G93A mice knock-out for TNFR2 were unchanged.

Discussion and conclusion: The results obtained indicate that the interaction between astrocytic TNFR2 and membrane-bound TNFa on neurons are involved in motoneuron death through a reverse signalling. They also show the contribution of TNFR2 expressed by motorneurons suggesting a new toxic mechanism involving TNFR2 expressed by both the cell types. However the lack of TNFR2 did not rescue SOD1-G93A mice knock-out indicating possible effects on the homeostasis of other biological systems, maybe protective, involved in the pathology. This aspect is under study. The comprehension of the mechanism involving TNFR2 can lead to innovative targets and strategies for therapeutical intervention.

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P64 REDUCED ASTROCYTE GLUTAMATERGIC AND TROPHIC RESPONSE TO ACTIVATION AND THEIR IMPLICATIONS ON THE PATHOGENESIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: reactive astrocytes, glutamate, neurotrophic factor

Background: Despite extensive efforts, the underlying cause of ALS and the path of neurodegeneration remain elusive. Astrocyte activation occurs in response to central nervous system (CNS) insult and is considered a double edged sword in many pathological conditions.

Objectives: We propose that reduced glutamatergic and trophic response of astrocytes to activation may, over time, lead to accumulative CNS damage, thus facilitating neurodegeneration.

Results and discussion: We found that astrocytes derived from the SOD1G93A 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 neurtrophic factor expression. However, not only do un-treated SOD1G93A 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 SOD1G93A astrocytes, improved their ability to protect the motor neuron cell line NSC-34 from glutamate induced excitotoxicity.

Conclusion: Our data indicates that altered astrocyte activation may well be pivotal to the pathogenesis of ALS.

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P65 NEURONALLY SECRETED ANGIOGENIN ACTS AS A PARACRINE FACTOR TO PROTECT MOTONEURONS IN ALS

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Keywords: angiogenin, RNA metabolism, paracrine

Background: The precise molecular and cellular basis for the neuronal death in ALS is not yet well established, but the contemporary view is that ALS results from a culmination of multiple aberrant biological processes. Among the proposed mechanisms of motoneuron degeneration, alterations on the homeostasis of RNA binding proteins and the consequent changes in RNA metabolism have also been implicated. Previous work (1) has shown that mutations in *angiogenin*, encoding a member of the pancreatic RNase A superfamily, segregate with both familial and sporadic cases of ALS. We also demonstrated that administration of angiogenin to

SOD1^{G93A} mice, a model of ALS, offers protection against motoneuron degeneration, both *in vitro* and *in vivo* (2, 3).

Objectives: In this work we sought to investigate the mechanism of angiogenin signalling and to characterize its role on motoneuron-astrocyte interactions.

Methods: Primary motoneuron cultures were established from spinal cord ventral horns from E13 C57 mouse embryos (4). Primary astrocyte cultures were prepared from cortices of P2 C57 black mouse pups.

Results: Using mixed motoneuron cultures, motoneuron-like NSC34 cells and primary astroglia cultures as model systems, we here demonstrate that angiogenin is a neuronally secreted factor that is endocytosed by astroglia and mediates neuroprotection in paracrine. We show that this uptake mechanism exists for mouse and human angiogenin, and delivers a functional RNase output. Angiogenin uptake into astroglia requires heparansulphate proteoglycans, and engages clathrin-mediated endocytosis.

Here we also identify syndecan 4 as the angiogenin receptor mediating the selective uptake of angiogenin into astroglia.

Moreover, we show that wild-type angiogenin acts unidirectionally to induce RNA cleavage in astroglia, while the ALS-associated K40I mutant is similarly secreted and endocytosed, but fails to induce RNA cleavage.

Angiogenin-induced fragments have been cloned and sequenced, and we are now validating these fragments as products of RNA cleavage mediated by angiogenin.

Discussion and conclusions: Our data provide new insights into the paracrine activities of angiogenin in the nervous system, and further highlight the critical role of non-neuronal cells in the pathogenesis of ALS. In addition, our results also raise the prospect that therapy with angiogenin or its products may prove efficacious in the treatment of ALS patients.

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P66 EFFECT OF BEE VENOM ON UBIQUITIN-PROTEASOME SYSTEM IN AN ALS MODEL

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Keywords: SOD1G85R, ubiquitin-proteasome system, bee venom

Familial ALS is caused by missense mutations in Cu, Zn-superoxide dismutase 1 (SOD1) that commonly result in the accumulation of mutant protein aggregates, which is associated with the impairment of the ubiquitin-proteasome system (UPS). UPS impairment is in turn implicated in many neurological disorders. Bee venom extracted from honey bees has been used as a traditional medicine for the treatment of inflammatory diseases and has been shown to attenuate neuroinflammatory events in a symptomatic ALS animal model.

To determine whether the mutant SOD1 gene affects UPS function in mutant-SOD1-expressing NSC34 cells, we transfected cells with a vector encoding the hSOD1G85R mutant, which is tagged with green fluorescent protein (GFP).

We found that hSOD1G85R overexpression induced SOD1 inclusions and reduced proteasome activity compared to overexpression of wild-type SOD1 in NSC34 motor neuronal cells. In addition, we observed that mutant SOD1 aggregate formation was increased by inhibition of proteasomal function using 10 mM lactacystin in hSOD1G85R-mutant-transfected NSC34 cells, suggesting that aggregate formation results from the defect in theUPS. To determine how bee venom regulates UPS function in hSOD1G85R-overexpressing NSC34 motor neuronal cells, we measured the proteasome activity and observed SOD1 inclusions. Interestingly, we found that bee venom enhanced proteasomal activity in hSOD1G85R-expressing neuronal cells and decreased the number of SOD1 inclusions compared to hSOD1-expressing NSC34 cells. Our findings suggest that bee venom could reverse the impairment of UPS function and reduce the neurotoxicity caused SOD1 inclusions in ALS models.

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P67 PROTEOMIC ANALYSIS OF ASTROCYTES AND CONDITIONED MEDIA FROM THE G93ASOD1 MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS REVEALS SECRETORY PATHWAY ALTERATIONS

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Keywords: astrocytes, SOD1, exosome

Background: The mechanisms leading to selective motor neuron death in amyotrophic lateral sclerosis (ALS) are still undefined. Recently, the interplay between motor neurons and glial cells, such as astrocytes, has been thoroughly investigated in mouse models of ALS, mice expressing mutant copper-zinc superoxide dismutase (SOD1). These studies have demonstrated that ALS is a non-cell autonomous disease, where the interaction between motor neurons and glial cells plays a role in motoneuronal degeneration and death. However, the molecules and pathways involved have not been identified yet.

Objectives: We analyzed the proteome and secretome of the astrocytes derived from G93ASOD1 mice, in comparison with those derived from mice over-expressing human wild-type (WT) SOD1, to identify possible differences induced by the expression of the mutant protein that could contribute to the disease.

Methods: Astrocyte cultures were prepared from 15–16-day-old mouse embryos. We used a 2DE-based proteomic approach to analyze the proteome and secretome of astrocytes. Proteins were identified by peptide mass fingerprinting and the identification of a selected number of proteins verified by immunoblot. Exosomes were isolated from conditioned media through ultracentrifugation.

Results: We identified 29 proteins differentially expressed in the G93ASOD1 astrocytes. The majority of the proteins play

an important role in secretory pathways, such as proteins of the endoplasmic reticulum and proteins involved in vesicle trafficking. The analysis of the conditioned media revealed that G93ASOD1 astrocytes globally secreted a lesser amount of proteins than WT SOD1 astrocytes. The secretome was very similar in the two situations except for nine protein. Among these there was human SOD1, and it was one of the proteins most up-regulated. Five out of nine proteins have been previously identified in exosomes. The amount of proteins in G93ASOD1-derived exosomes was substantially increased compared to WT SOD1, while the amount of SOD1 in the exosomal fraction is lower in the G93ASOD1 compared to WT SOD1 samples.

Discussion and conclusions: We observed a dysregulation of proteins involved in secretory pathways that ultimately lead to a general decrease of secreted proteins. This may activate compensatory mechanisms in astrocytes that could induce the activation of nonconventional secretion processes, leading to increased secretion of a selected number of proteins, including G93ASOD1. G93ASOD1 was found in exosomes but principally in exosome-depleted supernatants. This suggests that the mutation disfavors the incorporation of SOD1 into exosomes, but does not impede its increased secretion. It is possible to hypothesize that astrocytes, unlike motor neurons, are able to activate secretory pathways to eliminate mutant SOD1. This property could be at the basis of their ability to limit the formation of intracellular aggregates and to survive to mutant SOD1 toxicity, but on the other hand to ultimately exert a toxic effect on neighboring motor neurons.

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P68 THE ROLE FOR SELECTIVELY AUTOPHAGY IN ASC-J9-INDUCED NEURITE OUTGROWTH IN NSC34 CELLS THROUGH INHIBITION OF RHOA SIGNALING

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Keywords: ASC-J9, neurite outgrowth, autophagy

Background: Traumatic injury and neurodegenerative diseases are known to result in various deficits in neurite growth. Axons of adult central nervous system have very limited ability to regenerate after injury. This inhibitory effect is associated with inhibitory factors that create a non-permissive environment for regrowth after injuries. Recent studies suggest that these inhibitory molecules inhibit axon regeneration/ neurite outgrowth by activation of small GTPase RhoA and its downstream effector Rho kinase (ROCK).

Objectives: To investigate whether ASC-J9 can promote neurite outgrowth, growth cone extension and F-actin cytoskeletal remodeling in motor neuron-like cells (NSC-34 cells) and to explore the involved mechanisms.

Methods: To enhance the differentiation, differentiating medium comprising 1: 1 DMEM plus Ham's F12, 1% FBS and 1% modified Eagle's medium nonessential amino acids was changed when NSC-34 cells were grown to 60% confluence. Cells were allowed to differentiate for up to 5 days. NSC-34 cells were transfected with p62 siRNA using Lipofectamine 2000.

Results: ASC-J9 significantly induced neurite outgrowth of NSC-34 cells, even after the pre-induction of maturation. We have shown that ASC-J9 could activate selective autophagy. Here, we found that ASC-J9 treatment resulted in a significant decrease of RhoA expression. To further study the role of selective autophagy in ASC-J9-induced neurite outgrowth, NSC-34 cells were treated with ASC-J9 in the presence of 3-methyladenine (3-MA) (10mM) and bafilomycin A1 (10nM), inhibitors of selective autophagy, respectively. We found that not only 3-MA or bafilomycin A1 treatment but also p62 knockdown significantly inhibited the neurite outgrowth, extension of F-actin protrusions and reduction of RhoA expression mediated by ASC-J9 treatment.

Discussion: ASC-J9 treatment can up-regulate p62, the key component that regulate selectively autophagy. Our experiments indicate that selective autophagy also plays an important role in regulating RhoA signaling pathway. The exact mechanisms that p62-mediated selective autophagy affect the RhoA pathway remains to be elucidated.

Conclusions: ASC-J9 promotes neurite outgrowth in NSC34 cells, in which selective autophagy and RhoA inhibition might play an indispensable role.

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P69 ABNORMALLY ENLARGED AND DEFECTIVE ENDOSOMES ACCUMULATE EARLY AND SELECTIVELY IN MOTOR NEURONS OF ALS MODELS

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Keywords: endosomes, lysosomes, Rab5

Background: A common hallmark of ALS pathology is the aggregation of misfolded proteins in the cytoplasm of affected motor neurons. This accumulation suggests a mishandling of ALS-linked misfolded proteins and impairment of intracellular trafficking. The endosome-lysosome system (ELS) is a complex series of pathways that specifically drive the targeting of proteins for trafficking, degradation and exosomal secretion. Disruption of both exosomal secretion and protein degradation has been observed in ALS, suggesting a role of the ELS in ALS pathology.

Objectives: We hypothesised that ELS abnormalities will be common to misfolded ALS-linked proteins and will arise early in the disease course of ALS models.

Methods: NSC-34 cells stably expressing normal or mutant SOD1, TDP-43 or FUS were analysed for endocytic and lysosomal markers by Western blotting and immunocytochemistry. ELS markers were also examined in spinal cords of transgenic $SOD1^{G93A}$ and $TDP\text{-}43^{A315T}$ mice compared to wild-types by Western blotting and immunohistochemistry.

Results: We observed induction of early endosome (Rab5), late endosome (Rab7) and recycling endosome (Rab11) markers in NSC34 cells expressing mutant SOD1 and TDP-43 compared to wild-type forms. Rab5-positive endosomes were significantly enlarged (1–2 μm in diameter) with abnormal morphology in mutant SOD1 and TDP-43 expressing cells, compared to normal endosomes (200–400 nm). Rab5

expression was increased 2-fold in spinal cords of SOD1^{G93A} mice at 60 days of age, followed by 1.5-fold increases in Rab7 and Rab11 levels at 90 days. Immunohistochemical analysis supported these findings and revealed increased number and size of endosomes in presymptomatic SOD1^{G93A} mice in motor neurons, but not astrocytes or microglia.

Discussion and conclusions: Our findings show evidence of abnormal neuronal endosome accumulation and morphology which occurs early in the disease course of ALS models. These results implicate defects in endosome-lysosome trafficking in ALS which opens up new possible therapeutic targets.

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P70 ALS-LINKED MISFOLDED PROTEINS DISRUPT NEURONAL ENDOCYTIC TRANSPORT

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Keywords: endosome, transport, SOD1

Background: Cytoplasmic mislocalisation and accumulation of misfolded or mutant proteins, notably SOD1, TDP-43 and FUS, occurs in sporadic and familial ALS. The early involvement of endoplasmic reticulum stress and autophagy in ALS pathophysiology points towards defects in vesicle trafficking within motor neurons as central to cell susceptibility and death. The endosome pathway is a major intersection for intracellular vesicle trafficking in neurons and evidence linking mutations of *ALS2*, *CHMP2B* and *FIG4* to ALS supports a role for endosome abnormalities in ALS. We therefore sought to determine whether endocytic transport defects are common to key ALS-linked proteins.

Objectives: To investigate endocytic transport in cell models of ALS using tracers for receptor-mediated endocytosis and macropinocytosis.

Methods: NSC-34 cells were transfected with normal or mutant SOD1, TDP-43 or FUS and treated with the endocytic tracers, Alexa Fluor-conjugated epidermal growth factor (EGF) or transferrin (receptor-mediated endocytosis) or dextran (macropinocytosis). Cells were examined by confocal microscopy and immunolabelled for early, late or recycling endosomes.

Results: We observed a rapid uptake of EGF and transferrin in cells expressing wild-type SOD1, TDP-43 or FUS proteins within 30 min and recycling by 60 min. EGF and transferrin co-localised with markers of early endosomes. In contrast, EGF and transferrin internalisation was impaired in cells transfected with mutant SOD1, TDP-43 or FUS and a loss of co-localisation with abnormally enlarged endocytic vesicles was shown. Furthermore, mutant TDP-43 or FUS redistribution to the cytoplasm enhanced slowing of endocytic transport. Abnormal endosome transport was an early defect in cells preceding ER stress signalling, inclusion formation, autophagy and apoptosis.

Discussion and conclusions: Our results demonstrate that slowing of endocytic pathway trafficking occurs early in the toxicity of these ALS-linked misfolded proteins. Disruption of endosomal transport may also account for several other mechanisms implicated in neurodegeneration in ALS such as lysosomal activation and autophagy. We therefore propose that damage to endosome transport machinery or cargoes is an early and common feature in ALS pathogenesis.

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P71 ALS-ASSOCIATED PERIPHERIN ISOFORMS SHOW DYNAMIC INCLUSION MORPHOLOGY IN VITRO

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Keywords: inclusion, splicing, peripherin

Background: The intermediate filament protein peripherin has been detected within all sub-types of motor neuron inclusions found in amyotrophic lateral sclerosis (ALS), including Bunina bodies, ubiquitinated inclusions, hyaline conglomerate inclusions, and axonal spheroids. While the functional role(s) of these inclusions remain unknown, their formation may involve inter- and intra-isoform associations that arise through alternative splicing. An increase in peripherin mRNA that retains introns 3 and 4 (Per-3,4) leads to peripherin splicing abnormalities in ALS, including altered isoform stoichiometry and the generation of an aggregate-prone splice variant, Per-28.

Objectives and methods: During the course of evaluating Per-3,4 and Per-28 expression in SW-13 cells, we identified peripherin inclusions with categorically distinct morphology.

Results: Expression of the full-length peripherin isoform, Per-58, formed normal filaments in ~97% of transfected cells. In contrast, Per-3,4 expression was associated with cytoplasmic condensed/bundled filaments, small inclusions (< 10 μM), or large inclusions (≥ 10 μM) in ~34% of transfected cells, while Per-28 expression was associated with small punctate inclusions detected in the nucleus, cytoplasm, or both in ~93% of transfected cells. The addition of a C-terminal cMyc tag to each peripherin cDNA increased peripherin isoform-specific expression, but did not alter inclusion morphology. Temporal changes in inclusion formation and morphology were identified from 10, 24, and 48 hr transfected cells. Here, Per-3,4 and Per-28 showed up to 2.4- and 1.8-fold increases in inclusion formation at 24 and 48 hr when compared to 10 hr, respectively. This was accompanied by a 4.4-fold increase in large inclusions for Per-3,4 and a 1.4-fold increase in nuclear only inclusions for Per-28. Additionally, Per-3,4 inclusions were associated with disruption of endogenous vimentin networks, but not microtubules.

Discussion and conclusions: These observations may have relevance to understanding how peripherin-immunoreactive inclusions are formed in ALS. Further examination of the functional and pathological effects of peripherin splice variants *in vivo* is ongoing.

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P72 PRION MECHANISMS OF TOXICITY IN ALS: EXTRACELLULAR AGGREGATED WILD TYPE AND MUTANT G93A SOD1 INHIBIT ER TO GOLGI PROTEIN TRANSPORT AND ACTIVATES ER STRESS IN SH-SY5Y CELLS

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Keywords: prion-like, extracellular SOD1, ER stress

Background: The onset of Amyotrophic Lateral Sclerosis (ALS) is a focal process which spreads contiguously amongst upper and lower motor neurons. Recent studies have indicated that extracellular mutant SOD1 is involved in this process. Majorities (90%) of ALS cases are sporadic and misfolded wild type SOD1 is found in intracellular inclusions and in cerebrospinal fluid of sporadic patients. Little is known about the intracellular effect of misfolded forms of both extracellular wild type and mutant SOD1, and how their uptake could initiate neurodegeneration in motor neurons.

Objectives: ER stress is an important cellular process leading to cell death, and it occurs upstream in the pathogenesis of ALS. Recently our laboratory found that inhibition of ER to Golgi protein transport occurs as an early defect in cells expressing mutant SOD1, and this leads to the activation of ER stress. The aim of the current study is to investigate whether extracellular aggregated forms of mutant and wild type SOD1 activate ER stress by inhibiting ER to Golgi protein transport.

Methods: *In vitro* aggregation of recombinant wild type and mutant G93A SOD1 was performed, and the resulting proteins were labeled with Alexa Fluor® 488 dye and added to the culture medium of SH-SY5Y cells. ER to Golgi trafficking was examined using the cargo vesicular stomatitis virus glycoprotein ts045 (VSVG-ts045). Immunoblotting and immunocytochemistry techniques were used to detect upregulation of ER stress markers in these cells, and cell death was quantitated using propidium iodide (PI) uptake assay.

Results: Aggregated forms of extracellular wild type and mutant G93A SOD1, and native mutant SOD1, were taken up by SY-SH5Y cells. Aggregated wild type, native and aggregated mutant SOD1 specifically inhibited ER to Golgi protein transport to a similar degree. Furthermore, ER stress was also activated in these cells, determined by nuclear immunoreactivity to CHOP and upregulation of ER stress markers XBP1, IRE1 and BiP. Extracellular aggregated wild type and mutant SOD1 also induced cell death in comparison to untreated cells ($P < 0.01$).

Discussion and conclusion: This data shows that extracellular forms of both mutant SOD1 and aggregated wild type SOD1 induce ER stress and inhibit ER-Golgi transport similar to intracellular SOD1. These results therefore describe intracellular mechanisms leading to neurotoxicity triggered by extracellular mutant and wild type SOD1 after uptake. They also imply that wild type SOD1 is responsible for the spread of neurodegeneration in sporadic ALS. Hence therapeutic strategies targeting extracellular SOD1 could alleviate disease progression in both sporadic and familial ALS.

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P73 ENDOPLASMIC RETICULUM STRESS IN MURINE MOTOR NEURONE DISEASE INDUCED BY EXPRESSION OF HUMAN G93A MUTATED SOD1

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Keywords: unfolded protein response, endoplasmic reticulum stress, calcium homeostasis

Background: Endoplasmic reticulum (ER) stress seems to play a major role in the pathogenesis of familial and sporadic amyotrophic lateral sclerosis (ALS). When organelle bound calcium exchange dysregulates, ER stress occurs and misfolded proteins accumulate in the ER. To compensate for these changes the unfolded protein reaction (UPR) is activated.

Objective: To detect expression levels of key UPR signalling proteins in models of G93A hSOD1 amyotrophic lateral sclerosis.

Methods: Murine embryonic motor neurons were co-cultured on a prepared mono layer of astrocytes of the same Jackson black 6 mouse species with the same mutational status. These cultures, and in addition, G93A hSOD1 expressing NSC34 cells were exposed to several pharmacological stimuli to trigger ER stress. Western blot and immunohistochemistry was used to detect activation of several signal proteins indicating ER stress and the activation of the unfolded protein response.

Results: The UPR was activated in both cultured mutated SOD1G93A motor neurons and NSC34. Expression of XBP1 and ATF6α mRNA and protein was enhanced in SOD1G93A NSC34 cells. Activation of ATF6α, XBP1 and phosphorylation of eiF2α was detectable in mutated SOD1G93A motor neurones, but not in wild type motor neurones. Treatment with the ER stressor thapsigargin enhanced phosphorylation of eiF2α and expression of ATF6α, XBP1, and sXBP1 in NSC34 cells and motor neurons in a time dependent manner.

Discussion: Presently, the activation cascades known to occur in ER stress and UPR were found to be active due to hSOD1 expression. Notably, the increased level of the UPR was only mildly enhanced by known activators of the ER stress response in the presence of hSOD1. This provides further evidence that ER stress plays a major role in the presence of hSOD1, may exhaust the compensatory UPR prematurely and limit a motor neurone's ability to cope with additional physiological stress, eventually leading to motor neurone demise.

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P74 PROTECTIVE EFFECT OF PROTEIN DISULPHIDE ISOMERASE AND ITS MECHANISM AGAINST MUTANT SOD1 INDUCED TOXICITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: protein disulphide isomerase, protein disulphide isomerase family, superoxide dismutase 1

Background: Around 20% of familial amyotrophic lateral sclerosis (ALS) patients have superoxide dismutase 1 (SOD1) mutations and mutant SOD1 induces endoplasmic reticulum (ER) stress, inclusion formations and apoptosis in affected neurons. These processes are important hallmarks in the pathogenesis of ALS. Protein disulphide isomerase (PDI) is an important ER chaperone, which is also responsible for the formation and isomerisation of disulphide bonds. The disulphide interchange activity of PDI is due to the presence of cysteine residues present within its active sites. PDI is the prototype of a family of 19 members including PDIA2, ERp57, and ERp72, which possess structural similarities but different substrate specificities. We previously demonstrated that over expression of PDI has a protective effect against mutant SOD1 induced toxicity in ALS. However, the mechanisms underlying the protective functions of PDI, whether due to chaperone or disulphide interchange activity, are unclear.

Objective: To understand the mechanisms of action of PDI and other family members, including PDI, PDIA2, ERp57 and ERp72.

Methods: Mutants of PDI lacking the disulphide interchange activity were constructed, whereby active site cysteine residues were mutated to serine. PDI mutants, other family members PDIA2, ERp57, ERp72 and a small molecule mimic of the PDI active site, were also investigated, Constructs encoding PDI mutants and PDI family members were co transfected with mutant SOD1 into the motor neuron like cell line, Neuro-2a. Inclusion formations were examined by confocal microscopy, and ER stress and cell death was observed using immunocytochemistry and immunoprecipitation.

Results: Both PDI and ERp57 had a protective effect against mutant-SOD1induced toxicity whereas; PDIA2 and ERp72 had no effect, suggesting that the PDI family members are highly specific in their function and substrate associations. Furthermore, the PDI cysteine mutants did not decrease inclusion formations and ER stress whereas they all reduced apoptosis. These data suggest that the disulphide interchange activity of PDI is essential in decreasing inclusion formations and ER stress. However, the chaperone activity of PDI also plays a role in reducing cell death by other mechanisms.

Conclusion: PDI has an important physiological role in reducing mutant SOD1-induced toxicity. These data therefore reveal a possible mechanism through which PDI family members reduce this toxicity, thus revealing novel insights in the use of PDI and its family members as therapeutic agents in ALS.

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P75 UPREGULATION OF PROTEIN-DISULPHIDE ISOMERASE IN ALS MICROGLIA AND ER STRESS DEPENDANT ACTIVATION OF NADPH OXIDASE

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Keywords: glial cells, Protein disulphide isomerase, NADPH oxidase

Background: Glial cells play a major role in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Some familial forms of ALS are associated with point mutations in the superoxide dismutase 1 (SOD1) gene. However, the mechanism how mSOD1 causes direct and glia-mediated neurotoxicity is not fully understood. NADPH oxidase (NOX) activity and superoxide production have been shown to be elevated *in vivo* ALS studies, and they may thereby contribute the motoneuron death. Furthermore, mSOD1 has been demonstrated to bind to Rac1, a small G protein that activates NOX complex, and cause sustained NOX activation, leading to increased superoxide production. Misfolding and aggregation of mSOD1 protein overwhelms the protein folding capacity of the endoplasmic reticulum (ER) in neurons and induces the unfolded protein response (UPR), subsequently leading to apoptosis. Protein-disulphide isomerase (PDI) is an ER chaperone assisting oxidative protein folding in all types of cells, including neurons and glia. In neurodegenerative disorders, up-regulation of PDI is thought to be an important part of UPR and it is thought to protect the cells from apoptosis.

Objectives: In the light of the previous findings our aim was to explore 1) whether PDI expression is upregulated in glial cells of G93A-SOD1 mice as a part of UPR and 2) whether UPR results in PDI activity dependent NOX activation in microglia and other cells of monocyte lineage.

Methods: Early symptomatic, 20-week-old, G93A-SOD1 transgenic male mice carrying a high copy number of human mG93A-SOD1 were used for histological studies. Wild type (wt) littermates were used as control animals.

Results: In G93A-SOD1 ALS mice our results showed that even though PDI expression was high in motoneurons, the PDI positive area was not changed at early symptomatic age and we detected only slightly increased motoneuronal PDI expression when compared to wt mice. In contrast, we found a striking up-regulation of PDI in the spinal cord microglia of G93A-SOD1 mice, indicating that UPR may take place not only in neurons but also in glial cells in ALS. Because mSOD1 has been reported to cause sustained activation of NOX in microglia, we investigated the role of PDI and UPR in NOX activation. In BV-2 microglia UPR resulted in NOX activation coupled with increased superoxide production and TNF-α release. The phenomenon was recapitulated in primary rat microglia, murine macrophages and human monocytes. Importantly, pharmacological inhibition of PDI or its down-regulation by siRNAs prevented NOX activation in microglia and subsequent production of superoxide.

Discussion and conclusions: Our results show that increased PDI activity in microglia may exacerbate oxidative stress through NOX activation and thereby contribute to neurotoxicity, including the motor neuron degeneration in early stages of ALS.

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P76 IMPAIRED CYTOPLASMIC-NUCLEAR TRANSPORT OF HYPOXIA-INDUCIBLE FACTOR 1 IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: cytoplasm, nuclear transport, karyopherin

Background: Dysregulation of vascular endothelial growth factor (VEGF) is suggested to be part of pathogenesis underlying amyotrophic lateral sclerosis (ALS). Our previous study comprehensively examined cytokines/ chemokines in the cerebrospinal fluid (CSF) of ALS patients and revealed that reduced CSF levels of VEGF are associated with disease severity.

Objective: In the present study, we aimed to characterize underlying mechanisms for impaired VEGF production in ALS.

Methods and results: Immunohistochemical surveys on VEGF and upstream indispensable transcriptional factors, such as hypoxia-inducible factor-1a (HIF-1a), were performed using the anterior horn cells (AHCs) from postmortem ALS patients and G93A mutant superoxide dismutase 1 (mSOD1) transgenic mice, presymptomatic, symptomatic, and end stages, respectively. In ALS patients, the immunoreactivity of HIF-1a in the cytoplasm of AHCs increased significantly, while that of VEGF in the nucleus of AHCs decreased significantly compared with disease-free controls. In mSOD1-transgenic mice at the presymptomatic stage showed similar changes in the immunoreactivity of HIF-1a and VEGF. Furthermore, HIF-1a was co-localized with karyopherin b1 in the cytoplasm of AHCs from mSOD1-transgenic mice, and karyopherin b1 was co-localized with nucleoporin 62 on the nuclear envelope. Nuclear envelope alterations and changes in the immunoreactivity of karyopherin b1 occurred concurrently, presumable due to the impaired cytoplasmic-nuclear transport.

Conclusion: These results suggest that in the AHCs from mSOD1-transgenic mice, cytoplasmic HIF-1a is successfully transported to the nuclear envelope but cannot enter into the nucleus of AHCs as early as the presymptomatic stage. The impaired cytoplasmic-nuclear transport of HIF-1a might be a preceding event to motor neuron degeneration in ALS.

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P77 OVEREXPRESSED G93A HSOD1 ALTERS SENSITIVITY OF THE ENDOPLASMIC RETICULUM MITOCHONDRIA CALCIUM CYCLE TO MPTP ACTIVATION IN MOUSE EMBRYONIC MOTOR NEURONS

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Keywords: mitochondrial dysfunction, calcium dynamics, excitotoxicity

Background: In ALS, motor neurons display morphological abnormalities of mitochondria and the endoplasmic reticulum (ER) which are functionally coupled in the ER mitochondria calcium cycle (ERMCC). Decreased activity of mitochondrial complexes and the induction of the unfolded protein response (UPR) in sporadic ALS (sALS) patients and mutant hSOD1 mice indicate functional disturbances which may also be present in TDP-43 and VAPB models. Intervention studies showed that modulation of UPR sensor proteins has a life extending effect in mutant hSOD1 mice.

Objectives: To determine mitochondrial ERMCC kinetics and functional state in the presence of disease causing G93A hSOD1.

Methods: Mixed motor neuron cultures were prepared from E13 ventral spinal cord of non-transgenic and G93A hSOD1 mice. Cytosolic calcium transients and mitochondrial membrane potential were monitored using fura-2 AM and rhod123. ERMCC modifying drugs were tested for protective/toxic effects in motor neuron culture.

Results: Upon application of RU360, inhibiting the mitochondrial calcium uptake, motor neurons but not non-motor neurons failed to return kainate induced calcium levels to baseline. However, blocking of mitochondrial calcium export through the mitochondrial sodium/calcium exchanger (mNCE) by CGP37157 caused severely increased calcium transients in non-transgenic motor neurons but not in G93A hSOD1 motor neurons. Inhibition of the mitochondrial permeability pore (mPTP) with cyclosporine A had no effect on the cytosolic calcium dynamics, but a blocking of both ways of calcium extrusion with CGP37157 and cyclosporine A at the same time reduced intracellular calcium transients. Furthermore, 100 sec kainate exposure caused a steadily increasing loss of mitochondrial membrane potential in non-transgenic, but not in G93A hSOD1 motor neurons. A 12 h treatment with CGP37157 and CPA, reducing the calcium transport back to the ER showed a survival benefit for G93A hSOD1 motor neurons.

Discussion and conclusions: These results show that mitochondria indeed play a prominent role for the calcium buffering capability of non-transgenic motor neurons and G93A hSOD1 motor neurons, further revealing that the calcium uptake through the mitochondrial uniporter is not disturbed in the presence of G93A hSOD1. In contrast, mitochondrial calcium extrusion seems to be changed in G93A hSOD1 motor neurons. While the mPTP is not involved under physiological conditions, there seems to be an activation upon inhibition of the mNCE in non-transgenic motor neurons. Motor neurons of G93A hSOD1 mice showed no

compensatory mPTP activation. Furthermore, the evaluation of the mitochondrial membrane potential suggests an activation of the mPTP under severe stress conditions in non-transgenic motor neurons, but not in G93A hSOD1 motor neurons. First survival analyses in G93A hSOD1 motor neuron cultures were able to show, that ERMCC stabilization may provide a new therapeutic principle in ALS.

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P78 A PATHWAY CONTROLLING CELL SIZE AND AXON MORPHOLOGY OF MOTOR NEURON SUBSETS

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Keywords: motor neuron subset, vulnerability, motor axon

Background: Subsets of motor neurons greatly differ in morphology but the developmental programs regulating the size of their cell bodies, axons and dendrites remain unclear.

Objective and methods: To address this, we FACS-isolated two distinct motor neuron subsets innervating either limb muscles (LMC-MN) or axial muscles (MMC-MN) and investigated them by morphometric, gene profiling and functional approaches.

Results and discussion: We demonstrate that repression of the transcription factor Hb9 in LMC motor neurons causes strong up-regulation of the intermediate filament protein Peripherin and the enzyme Diacylglycerol kinase β which both contributes to the larger cell bodies and longer but less branched axons in LMC as compared to MMC motor neurons.

Conclusion: This data might provide molecular clues to the differential vulnerability of motor neurons in degenerative motor neuron diseases such as ALS and SMA.

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P79 GFP-BASED REPORTERS TO MONITOR BIOCHEMICAL ALTERATION IN CELLULAR MODELS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: high-throughput experimental strategies, oxidative stress, ubiquitine-proteasome system

Background: A critical step in designing more effective therapies for the treatment of ALS, is the identification of drugs able to interfere with the first cellular alterations, prior to the onset of motoneuron death. High – throughput experimental strategies (HTS) aimed at the finding of pharmacological compounds that might slow, stop or reverse disease progression may provide a unique and powerful tool for the identification of new active drugs for treating ALS.

Objectives: Development and validation of different cellular models, where specific markers of cell toxicity induced by expression of ALS-causative genes, may be used as a straightforward read-out for screening of compound libraries of different nature. In particular, we validate two different cellular models expressing high sensitive fluorescent probes suitable for the measurement of either oxidative stress or proteasomal impairment, which have been described to be key events during ALS disease progression.

Methods: We generate cell lines expressing constitutively the following fluorescent reporter genes to analyze and quantify *in vivo* those parameters shown to be altered in ALS: the oxidative stress (roGFP reporter) and the ubiquitine-proteasome (UPS) activity impairment (YFPu reporter).

a) roGFP senses the redox potential of the cellular glutathione buffer. Neuronal SHSY5Y cells stably expressing roGFP or mitochondrial targeted roGFP (MTXroGFP).

b) To measure the UPS function in living cells, we took advantage of a reporter consisting of a short degron, CL1, fused to the C–terminus of the Yellow Fluorescent Protein (YFPu) whose signal has been demonstrated to be quickly detectable in living cells in case of UPS impairment.

Recombinant adenoviruses encoding wild type or mutant (G93A or H80R) SOD1 were generated according to manufacturer's instruction (Clontech).

Results: Infection of SH-SY5Y cells, expressing either MTXroGFP or YFP-u, with adenoviruses encoding SOD1 WT and two different mutants (G93A or H80R), indicate a significant increase in roGFP and YFPu fluorescence following infection with SOD1 mutant, compared to SOD1-WT, suggesting that these reporters permits real-time analysis of cell damage triggered by ALS-causative genes, in living cells.

Discussion: Although preliminary our results indicated that both cellular models are able to analyze and measure *in vivo* the cellular stress induced by the ALS-causative gene SOD1. Studies are in progress to verify suitability of different cellular models for the HTS analysis to identify biological active molecules able to interfere with and/or prevent cellular damage generated upon expression of ALS causative–genes.

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THEME 4 HUMAN CELL BIOLOGY AND PATHOLOGY

P80 NEURODEGENERATION-ASSOCIATED TDP-43 INTERACTS WITH FMRP/STAU1 AND REGU-LATES SIRT1 EXPRESSION IN NEURONAL CELLS

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Keywords: TDP-43, FMRP, STAU1, SIRT1

Background and objective: Despite the identification of the 43kDa transactive response DNA-binding protein (TDP-43) as a major pathological signatory protein in a wide range of neurodegenerative diseases, the mechanistic role of TDP-43 in neurodegenerative disorders is still poorly understood.

Methods and results: We report that TDP-43 is physically associated with fragile X mental retardation protein (FMRP) and Staufen (STAU1) to form a functional complex. Differential microarray analysis revealed that the expression of a collection of functionally important genes including Sirtuin (SIRT1) is regulated by this complex. RNA-immunoprecipitation (RIP) assays demonstrated that TDP-43/FMRP/STAU1 specifically bind to the 3'UTR of SIRT1 mRNA, leading to the degradation of SIRT1 mRNA. SIRT1 is implicated in double-stranded DNA break repair and is required for cell survival. Indeed, depletion of TDP-43/FMRP/STAU1 sensitizes cells to apoptosis and DNA damages.

Conclusions: Our experiments revealed that TDP-43 is physically associated with FMRP and STAU1 to form a functional coordinated complex to co-regulate the expression of SIRT1. Our results indicate that defects in the TDP-43/FMRP/STUA1 complex sensitize neuronal cells to apoptosis and DNA damages in a SIRT1-dependent manner. These findings may shed new light on the understanding of the biological functions and the mechanistic involvement of TDP-43 in neurodegenerative diseases.

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P81 INTRINSIC REGULATION OF FUS SUBCELLULAR LOCALIZATION AND AGGREGATION BY DIFFERENT SEQUENCE FEATURES

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Keywords: FUS, subcellular localization, protein aggregation

Background: Mutations of DNA/RNA binding protein fused in sarcoma/translated in liposarcoma (FUS/TLS) have been recently reported to be responsible for the familial ALS type 6. In neurons, FUS is predominantly a nuclear protein and can shuttle between the cytoplasm and nucleus. Mutated FUS is found to accumulate in the cytoplasm and forms cytoplasmic inclusion in postmortem ALS patient brains. Most of the mutation in FUS is clustered at the C-terminus. The very C-terminus of FUS has been identified to be an effective nuclear localization sequence (PY-NLS), and ALS related C-terminal mutation will compromise the function of nuclear localisation signal (NLS). However, the sequence features determining the subcellular localization of FUS and their implication in the aggregation are not fully understood.

Objectives: To study the sequences involved in the import or export of FUS and their relevance to the aggregation.

Methods: Different mutations or truncations of FUS were made and expressed in neuroblastoma 2A in the presence or absence of export 1 specific inhibitor LMB, and confocal images were took to observe their localization. Mouse primary motor neurons and skin fibroblast cells derived from the familial ALS patients carrying FUS mutations were used in the study to validate the findings from the N2A cells.

Results: We found sequence 301VADYFKQIGI310 is a functional nuclear export signal (NES). In addition to the C-terminal NLS, the RRM domain in the C-terminus may play a functional role of nuclear retention, and N-terminus can also direct the nuclear import of FUS through an unknown mechanism. FUS only forms aggregates in the nucleus, and C-terminus of FUS including RRM domain is critic but not sufficient for the aggregation of FUS.

Discussion and conclusions: The import and export of FUS is regulated by multiple domains. Cytoplasmic localization is critic for the aggregation of FUS. Our data suggest mutated FUS can still shuttle between nucleus and cytoplasm. Further study might identify the underlying molecules driving the shuttling of FUS and develop therapeutic strategy.

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P82 ARGININE METHYLATION REGULATES FUS CHROMATIN-ASSOCIATION AND SOLUBILITY

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Keywords: FUS, arginine methylation, protein solubility

Background: Mutations on RNA binding protein FUS account for 4–5% of familial ALS. How FUS mutants cause motor neuron degeneration is unknown. FUS toxicity is closely related to its intracellular localization and extracellular solubility.

Objectives: To understand FUS proteinopathy by studying the regulation of its localization and solubility.

Methods: We transfected HEK 293T cells with plasmid expressing GST-tagged FUS or FUS mutants and did cell fractionation assay to detect the intracellular localization of FUS and FUS mutants. We transfected HEK 293T cells with plasmid expressing GST-tagged FUS and treated the cells with methyltransferase inhibitor to investigate the effect of arginine methylation on FUS localization and solubility. We cotransfected HEK 293T cells with plasmid expressing GST-tagged FUS and plasmid expressing EGFP-tagged TDRD3 (Tudor domain-containing protein 3) to study FUS-TDRD3 interaction and localization.

Results: FUS is predominantly nuclear-located and presents in two fractions: nuclear soluble and chromatin associated. We found ALS-related mutations dissociate FUS from chromatin. FUS with low arginine methylation level tends to accumulate in the chromatin fraction, suggesting arginine methylation facilitates FUS chromatin dissociation. Methylated arginine is a binding substrate of protein tudor domain. In our study, we found FUS interact with TDRD3 and this interaction was weakened by methyltransferase inhibitor treatment. Overexpression of TDRD3 promoted FUS chromatin dissociation and FUS mutants cytoplasmic accumulation. Moreover, we found ALS-related mutations and low arginine methylation level reduces FUS solubility in radioimmunoprecipitation assay (RIPA) buffer.

Discussion and conclusions: Our findings suggest that arginine methylation is an important post-translational modification in the regulation of FUS localization and solubility. Manipulation of FUS arginine methylation level can be applied to reduce FUS toxicity.

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P83 HISTONE DEACETYLASE 6 REGULATES FAMILIAL ALS MUTANT SOD1 TURNOVER

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About 20% of the familial ALS cases are caused by mutations in the Cu/Zn superoxide dismutase (SOD1). Protein inclusions in motor neurons and glial cells are a hallmark of ALS and the ALS mutants of SOD1 are prone to aggregation. HDAC6 is a lysine deacetylase that is almost exclusively localized in the cytoplasm. HDAC6 was implicated in the regulation of protein aggregation and autophagy. The role of HDAC6 in ALS has not been investigated in detail.

We found that the knockdown of HDAC6 resulted in the accumulation of highly aggregated mutant SOD1. HDAC6 selectively interacted with mutant, but not with wild-type SOD1. The ubiquitin-binding Zn-finger and the lysine deacetylase activity of HDAC6 were not necessary for the HDAC6-mutant SOD1 interaction. Deletion of the SE14 repeat domain of HDAC6 impaired the interaction with mutant SOD1. However, the isolated SE14 domain did not interact with mutant SOD1. Instead, we found that a motif very similar to the SOD1 Mutant Interaction Region (SMIR) we described in p62/Sequestosome 1 is present near the SE14 domain in HDAC6 and the SMIR mediates the interaction with mutant SOD1. We also identified a second, less conserved SMIR in the first deacetylase domain of HDAC6. We also found that p62 and HDAC6 interacted with each other and this interaction was at least in part mediated by the SMIR motifs of p62 and HDAC6.

Our results suggest that beyond their functional interaction in the regulation of protein aggregation and turnover, HDAC6 and p62 can also participate in common biochemical complexes and share a mechanism to recognize mutant SOD1 independent of their ubiquitin-binding domains. We also investigated the modulation of the tubulin deacetylase activity of HDAC6 by mutant SOD1 and the possible role of tubulin acetylation in mutant SOD1 turnover.

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P84 ALTERATED EXPRESSION OF SOD1 AND TDP-43 IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM SPORADIC ALS PATIENTS

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Keywords: SOD1, TDP-43, peripheral blood mononuclear cells

Background: Several lines of evidence supported the hypothesis of a toxic role played by wild type (WT-SOD1) in the pathogenesis of SALS (1–2). Nevertheless, TDP-43 has been identified as the major pathological protein due to its mislocalisation in the cytoplasm (3). As recent studies (4,5) support the presence of a relationship between these two proteins here we aim to investigate SOD1 and TDP-43 expression in peripheral blood mononuclear cells (PBMC) from SALS patients.

Objectives: Evaluate SOD1and TDP-43 expression and localization in lymphocytes and monocytes from SALS patients.

Methods: PBMCs were obtained from 20 SALS patients and 20 healthy controls (CTR). Nuclear and cytoplasmic fractions were obtained as previously described by (6). Insoluble pellets were resuspended in lysis buffer as reported by (7). SOD1 and TDP-43 expression were evaluated by western blotting. SOD1 localization was evaluated by immun-

ofluorescence technique followed by confocal microscopy observation.

Results: Western blot analysis showed an overall increased nuclear SOD1 expression in SALS patients. The splitting of patients with higher nuclear SOD1 expression from those with similar to controls' levels highlighted the differences between the patients' clustering. No differences were found in the cytosolic compartment. Analysis of insoluble lysates evidenced an increase in SOD1 expression in a group of ALS cases suggesting that a reduction in SOD1 expression in some patients was due to the protein precipitation in the insoluble fraction. A further indirect confirmation of these finding was obtained by immunofluorescence and confocal analysis. The presence of small or large foci of aggregated SOD1 protein was regularly detected in the cytoplasm of patients with nuclear SOD1 levels similar to controls. Patients with high nuclear SOD1 levels showed smaller aggregates in the same compartment. Regarding TDP-43, we observed higher nuclear levels compared to cytoplasm in both SALS patients and control, confirming its distribution predominantly in the nuclear compartment. Interestingly, 6 patients with high levels of nuclear SOD1 showed also high level of cytoplasmic TDP-43.

Conclusions: These preliminary data suggest us the involvement of an altered WT-SOD1 and TDP-43 localization in sporadic cases of ALS highlighting new evidences about pathways which may have implication in the disease pathogenesis.

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P85 GENE EXPRESSION PROFILING OF LYMPHOBLASTOID CELLS FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH AND WITHOUT C9ORF72 EXPANSIONS AND CONTROLS

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Keywords: transcriptomics, C9ORF72, RNA processing

Introduction: Intronic hexanucleotide repeat expansions of C9ORF72 are found in approximately 10% of patients with amyotrophic lateral sclerosis (ALS) and represent the most common genetic variant identified to date. The function and dysfunction of C9ORF72 is poorly understood, but it has been proposed that the expansion may mediate pathogenesis via a sequestration of RNA splicing factors.

Metholodology: Gene expression profiling (GEP) was carried out on RNA extracted from lymphoblastoid cells from ALS patients with the C9ORF72 expansion (n = 10), ALS patients without the expansion (n = 16) and neurologically normal controls (n = 10) using Affymetrix HG-U133 Plus 2.0 GeneChips. Data were analysed with the Propagating Uncertainty in Microarray Analysis (PUMA) suite of tools. Aberrantly affected pathways were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Differential expression of certain genes was validated by QRT-PCR. A multiplexed fluorescent bead-based immunoassay of cerebrospinal fluid (CSF) from C9ORF72-ALS (n = 4), non-C9ORF72 ALS (n = 5) and controls (n = 5) was used to confirm changes in gene expression at the protein level.

Results: 319 probe sets were differentially expressed identified between C9ORF72-ALS cases and controls. Significantly enriched pathways included 'RNA splicing' and 'chromatin modification' ($p < 0.01$). Consistent with earlier studies, C9ORF72 itself was down-regulated. Successful validation of gene expression changes was performed by QRT-PCR. The top differentially expressed gene, as determined by fold change, between C9ORF72-ALS and controls was down-regulation of a neuroprotective cytokine, which was also the second highest differentially expressed gene between C9ORF72-ALS and non-C9ORF72 ALS. A lower level of this cytokine in C9ORF72-ALS compared to non-C9ORF72 ALS ($p < 0.05$) and controls was confirmed in CSF.

Discussion: Identified down-regulation of splicing factors is consistent with sequestration which may mediate a broader disruption of RNA splicing. As other splicing associated genes were up-regulated, it is hypothesised that this represents compensation and a potential therapeutic target. A similar up-regulation of these genes has been discovered in frontotemporal lobar degeneration with TDP-43-positive inclusions (FTLD-TDP). Certain results were comparable to gene expression in myotonic dystrophy 1 (DM1), another neuromuscular disease mediated by an intronic expansion. The extent to which disease mechanisms in C9ORF72-ALS and DM1 are similar remains to be determined. The identification of aberrant down-regulation of a neuroprotective cytokine in C9ORF72-ALS compared to non-C9ORF72 ALS and controls suggests that pathways of inflammation are important in the pathogenesis of C9ORF72-ALS. This may represent a novel therapeutic target.

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P86 ANALYSIS OF THE C9ORF72 HEXANUCLEOTIDE REPEATS SIZE IN ALS PATIENT DERIVED FIBROBLASTS, IPS CELLS AND POSTMORTEM BRAIN TISSUES

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Keywords: hexanucleotide repeat, C9ORF72, southern blotting

Background: Approximately 10–15% of ALS cases are familiar. Mutations in *SOD1* encoding the cooper/zinc superoxide dismutase, *TARDBP* (TAR DNA-binding protein 43) and *FUS* (fused in sarcoma) are responsible for 20%–30% of familial ALS disease. Rare mutations in other genes like *ANG*, *VAPB, DAO*, *OPTN*, *VCP* and UBQLN2 have been reported. Recent studies have identified expanded GGGGCC hexanucleotide repeats in the C9ORF72 gene in ALS, frontotemporal dementia (FTD) and ALS-FTD. This novel mutation is currently the most common genetic cause of familial ALS (23%–47%) as well as sporadic ALS (4%–21%) in the USA, Belgium and Finland. Characterization of the role of repeat length and tissue distribution effects of variation in repeat expansion size in the *C9ORF72* gene is suggested to be crucial for the understanding of the mechanisms and pathways of ALS pathology.

Objectives: ALS patient derived fibroblasts(3), iPS cells(3) and postmortem brain tissues(6).

Methods: The size and number of repeats were determined by optimized Southern Blotting approach.

Results: All the expanded repeats are over 3 kb long and one of them was found to contain over 1000 repeats. Interestingly, the size and number of repeats in the iPS cell lines were different from the fibroblasts derived from the same patients. Repeat length in our control group close to the findings of previous reports. We also examined the dynamic changes in the repeat size as cell lines underwent increasing passages. Finally we analyzed repeat length in multiple different brain region from more than six C9ORF72 autopsies.

Discussion and conclusions: *Southern Blotting is the only approach to determine GGGGCC hexanucleotide repeats in the C9ORF72 gene once the repeat number is over 100 since it is 100% GC rich region. The size and number of repeats in the iPS cell lines were different from the fibroblasts derived from the same patients based on our result*. Here we report data on the repeat size in the gene in different cell types and tissue samples obtained from ALS patients. Using an optimized Southern Blotting approach, the size and number of repeats were determined in fibroblasts and iPS cell lines derived from ALS patients with C9ORF72 repeat mutations.

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P87 MICROARRAY ANALYSIS IN SPINAL CORDS OF SPORADIC ALS PATIENTS WITH CELL-TYPE SPECIFIC TRANSCRIPTOME

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Keywords: microarray, non-cell autonomous neuronal death, glia

Objective: With DNA microarray, we analyzed the molecular pathomechanism in sporadic ALS spinal cords with a focus on the function of microglia and astrocytes. We analyzed the microarray data in a cell type specific manner to understand the molecular mechanisms within each cell type of ALS spinal cord.

Background: Glial cells including astrocytes and microglia are reported to be actively involved in motor neuron death in ALS, but the precise mechanisms for the 'non-cell autonomous neuron death' have not been elucidated.

Design and methods: We profiled using DNA microarray the mRNA expression with cervical spinal cords of 4 sporadic ALS patients and 5 disease-control. To predict the cell type(s) in which each gene was expressed abundantly, we established the cell-type specific transcriptomes using mouse CNS primary culture, then the integrated database was converted to human orthologue. Isolated misregulated genes from microarray were analyzed in terms of glial functions, by using cell-type specific mouse transcriptomes.

Results: We isolated over 200 genes which were significantly changed in the spinal cords of ALS patients. We then classified these genes according to the cells that expressed those genes abundantly, and found nearly half of those genes were expressed abundantly in microglia or astrocytes. Furthermore, many of these genes were also changed in ALS mouse models ($SOD1^{G37R}$ Tg mice, $SOD1^{G85R}$ Tg mice). We confirmed that the predicted gene expression pattern was true by immunohistochemistry for several genes with spinal cords of ALS mouse models. Pathway analysis predicted that innate immunity was one of the significantly altered pathways in glial cells.

Conclusion: We could predict the molecular pathomechamism, especially of glia contributing to the non-cell autonomous motor neuronal death of the ALS in the spinal cord that consists of heterogenous cell types.

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P88 REDUCED LEVELS AND ALTERED SUBCELLULAR DISTRIBUTION OF THE INSULINOMA-ASSOCIATED PROTEIN 2 (IA-2) IN ALS AND FTLD

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Keywords: endosomes, membrane trafficking, neuronal vulnerability

Background: Several mutations linked to ALS implicate dysregulated endosomal trafficking as an important pathophysiological mechanism. Changes in the expression of constituents

of the regulated secretory pathways in neurons may contribute to selective vulnerability not only in ALS but also in frontotemporal lobar dementia (FTLD). We recently used unbiased proteomics methodologies to identify proteins that exhibit abnormal levels in the CSF of ALS patients. We detected reduced Insulinoma-associated protein 2 (IA-2) in the CSF of ALS patients. IA2 is a transmembrane protein within the protein tyrosine phosphatase superfamily that appears enriched in the secretory vesicles and dense-core granules of various neuroendocrine cell types including central nervous system (CNS) neurons. IA-2 is a major autoantigen in type I diabetes mellitus (T1DM).

Objectives: To characterize the expression and distribution of IA-2 in ALS and FTLD to identify novel mechanisms related to disregulated endosomal trafficking in ALS and FTLD.

Methods: Paraffin tissue and snap frozen tissue from the spinal cord, hippocampus, and frontal cortex of ALS, FTLD, and age-matched control patients were subjected to IA-2 immunohistochemistry (IHC) and immunoblot analysis.

Results: Immunohistochemistry demonstrated decreased IA-2 in spinal cord motor neurons of ALS patients, consistent with our prior proteomic analysis. Furthermore, we observed by immunoblot the loss of the full-length IA-2 isoform in spinal cord tissue from ALS patients compared to controls. In addition, IA-2 was present in distal neuritic processes and neurites in the frontal cortex and hippocampus from FTLD patients.

Conclusions: Our results suggest that reduced levels of IA-2 in ALS (displayed by both IHC and mass spectrometry) and altered subcellular distribution of IA-2 in neurites of FTLD further indicate dysregulation of endosomal trafficking in these neurodegenerative disorders. Moreover, we observe a loss of the full-length IA-2 isoform in ALS. Ongoing studies will clarify whether the loss of full length IA-2 is due to altered mRNA processing or to protein catalysis. Future studies will explore how these changes in IA-2 are related to neuronal loss, and if autoimmune mechanisms resembling T1DM are involved. Motor neurons and cortical motor neurons may be particularly susceptible to this failure because their long axonal processes result in a high demand for turnover of membrane components.

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P89 EVALUATION OF TH1/TH2 LYMPHOCYTE BALANCE AND MACROPHAGE SCAVENGER RECEPTOR EXPRESSION IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS)

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Keywords: alternative immune activation, CD36 scavenger receptor, Th1/Th2 balance

Background: Involvement of classical monocyte/macrophage (MO) activation and inflammation in ALS pathogenesis has been confirmed by various investigations. Our recent studies on gene expression in peripheral blood mononuclear cells from patients with ALS showed upregulation of both classic type I (M1) interferon-induced genes and type II (M2) alternative MO activation genes, suggesting a hybrid activation state that implicates both classical and alternative MO activation in ALS pathogenesis. Abnormal elevation of alternative MO activation-associated chemokine CCL18 was also observed in ALS plasma. In an attempt to provide further evidence of alternative MO activation in ALS, we evaluated expression of macrophage scavenger receptor CD36, an alternative MO activation marker, and balance of T-helper 1 (Th1) and Th2 subsets of CD4 + lymphocytes in sALS patient blood. Th1 and Th2 cells can be redefined as polarized forms of immune responses; mirroring the Th1/Th2 nomenclature, many refer to polarized macrophages as M1 and M2 cells.

Objectives: To assess CD36 expression on circulating MO and Th1/Th2 lymphocyte markers in sALS patients and healthy controls, and determine whether levels of systemic alternative immune activation correlated with degree of classic MO activation, and clinical stage of disease and/or riluzole treatment in ALS.

Methods: Flow cytometry was performed to examine blood MO CD36 expression and Th1/Th2 lymphocyte balance in heparinized blood samples of 40 sALS patients and 36 healthy controls (HC). Results from immune studies were analyzed in relation to riluzole treatment and the severity of neurological impairment as determined by ALSFRS-R score.

Results: Patients with sALS had significantly elevated levels of CD36 expression on blood MO as compared to healthy controls (HC, 33.52 ± 24.15; sALS, 48.85 ± 27.27; $p = 0.0083$). Analysis of Th1/Th2 lymphocyte markers showed that Th2 marker of CD4 + CCR4 + was significantly higher (HC, 31.01 ± 7.23; sALS, 39.49 ± 9.58; $p < 0.0001$), and CD4 + CXCR3 + /CD4 + CCR4 + ratio (representing Th1/Th2 balance) was lower (HC, 1.24 ± 0.42; sALS, 1.04 ± 0.29; $p = 0.0232$) in sALS patients than healthy controls. No relationship was found between MO CD36 expression and classic type I MO activation in ALS. MO CD36 expression and Th1/Th2 imbalance in sALS patients were independent of severity of disease, and did not change with riluzole therapy.

Discussion and conclusions: The current study showed persistent disease-associated increases of MO CD36 expression and imbalance of Th1/Th2 lymphocytes in sALS. Considering that a systemic Th2 shift is part of a necessary central nervous system wound healing reaction after injury, MO alternative activation and Th2-cell anti-inflammatory response in ALS may represent a negative feedback mechanism in modulation of an inflammatory response, limiting the propagation of classical type I MO activation and Th1 inflammatory responses and compensating for the inflammatory compounds in ALS disease process. Further investigation will be needed to characterize the role of alternative MO activation and Th1/Th2 shift in ALS pathogenesis.

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P90 NORMAL LEVELS OF GLUCOSE PROTECT FIBROBLASTS FROM PATIENTS WITH ALS BY REDUCING ROS PRODUCTION AND MAINTAINING THE EXPRESSION OF ROS-DETOXIFYING ENZYMES

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Keywords: glucose, hypermetabolism, reactive oxygen species

Background: There are many mechanisms involved in the pathophysiology of amyotrophic lateral sclerosis (ALS): glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, etc. There are also increasing evidences that the disease has systemic features beyond the nervous system. In fact, abnormalities of carbohydrate metabolism and insulin resistance have been reported in patients with ALS since the 1980s (1). Moreover, a state of hypermetabolism has been documented, probably related with a defective homeostasis of glucose in muscle tissue (2). We have earlier reported that fibroblasts from patients growing in low glucose medium had a significant increase in the activity of mitochondrial respiratory chain complexes, when compared with control cells or with fibroblast from patients growing in high glucose concentration. In low glucose medium, cell viability and duplication time were similar to those of control cells. However, a significant reduction in cell viability was observed when they were grown in a medium with high glucose levels. We proposed that the hyperactivity of the respiratory chain allows to cells maintain their mitochondrial membrane potential and survival (3).

Objectives: Because the increase of radical oxygen species (ROS) production is a hallmark of cellular dysfunction in ALS, our aims are to study how glucose affects ROS generation in cells growing in different concentration of the substrate and determine if there are changes in the expression of genes encoding ROS detoxifying enzymes.

Methods: Skin fibroblast cultures were established from age-matched controls and patients ($n = 10$ and $n = 6$, respectively) after their informed consent. ROS production was measured with the fluorescent dye H_2DCFDA in cells growing in a medium with low glucose (1 g/L) or high glucose concentration (4.5 g/L) as described (4). Expression of detoxifying enzymes was determined by RT-PCR as reported (5).

Results: Fibroblast from patients had an increased ROS production in low and high glucose medium (1.5-fold, $p < 0.01$ and 2.2-fold, $p < 0.01$, respectively) when compared with control cells. Moreover, there was a significant difference between patients' cells when were grown in low or high glucose concentration media ($p < 0.01$). There were no differences between expression levels of CuZnSOD, MnSOD, catalase and GPx in control cells when were cultured in both mediums. However, there was a significant reduction in the expression of CuZnSOD and catalase ($p < 0.01$) in cells from patients when were grown in high glucose concentration.

Conclusion: A low level of glucose reduces ROS production and allows the fibroblasts from patients with ALS to maintain normal expression of ROS-detoxifying enzymes.

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P91 EFFECTS OF ANTIOXIDANT AGENTS ON MITOCHONDRIAL FUNCTION AND CALCIUM HOMEOSTASIS IN SKIN FIBROBLASTS OF PATIENTS WITH SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mitochondria, antioxidants, calcium

Introduction: Amyotrophic lateral sclerosis (ALS) has been commonly regarded as a neurodegenerative disorder primarily involving the pyramidal motor system. There is however some evidence that disease-related degenerative changes also occurs in extraneuronal tissues. Therefore, primary skin fibroblast cultures from ALS with mutations in the SOD1 gene were compared with ALS patients without mutations and healthy controls for mitochondrial anomalies.

Objective: To verify the putative impairment of mitochondrial function in extraneuronal tissue of patients with sporadic or familiar ALS, the oxygen consumption (respiration rate) of fibroblasts was measured using a high resolution oxygraph. Furthermore, putative benefits of antioxidative agents on the mitochondrial function were assessed.

Methods: Flux control analysis was applied for quantification of changed respiratory chain complexes. To check possible metabolic consequences for calcium homeostasis fluorimetric measurement of cytosolic Ca^{2+} concentrations were performed. Furthermore, mitochondrial DNA copy number analysis and deletions screening were performed. Age matched healthy subjects served as controls.

Results: Enzymatic and respirometric measurements clearly demonstrate mitochondrial impairments in sALS fibroblast with complex I as the main target. Although we have so far investigated only mitochondria of 5 fALS patients, it seems that the extent of complex I impairment is less than as in sALS mitochondria. In both, sALS and fALS patient groups the Ca^{2+} release into the cytosol induced by histamine and FCCP was significantly diminished. Furthermore we detected, that decreased as well as elevated stationary basal Ca^{2+} concentrations could occur in ALS fibroblasts indicating differences in the pathophysiological mechanisms of sALS and fALS. Under endogenous conditions the enlarged Ca^{2+}_{cyt} (sALS) could cause an over-energization and the diminished Ca^{2+}_{cyt} at fALS could cause an under-energization of mitochondria with consequences for the requirements of intact cells. Most interestingly, the antioxidants Trolox and CoQ_{10}

ameliorate the functional impairment of mitochondria from both groups of patients, most probably by a direct anti-oxidative and membrane-stabilizing action on mitochondria. No significant differences in mtDNA copy number or common deletion level between patients and healthy controls were found.

Conclusions: Our results support the viewpoint that mitochondrial impairments and consequently Ca^{2+}-dyshomeostasis are detectable in extraneuronal tissues of patients with ALS. Fibroblasts may serve as a relatively easily accessible read out system to test the anti-oxidative properties of specific substances in ALS.

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P92 CELL CYCLE DYSREGULATION IN MOTOR NEURONS OF SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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Keywords: androgen receptor, TGF-beta, cell cycle

Background: Our previous study showed that the poly-glutamine-expanded AR protein, the causative protein of spinal and bulbar muscular atrophy (SBMA), induces transcriptional dysregulation of type 2 TGF-beta receptor and thereby inhibits TGF-beta signalling (1). Although TGF-beta signaling is known to regulate a diverse range of cellular responses including cell cycle, it is unclear how the disruption of this signaling causes neuronal dysfunction in SBMA.

Objectives: The aim of this study is to elucidate the molecular events downstream to the disruption of TGF-beta signaling, and identify the signal pathway that leads to neuronal cell death in SBMA.

Methods: Expression of cell cycle-specific proteins in motor neurons of a transgenic mouse model of SBMA (AR-97Q) was investigated using immunohistochemistry. The effects of pharmacological inhibition of TGF-beta on cell cycle were analyzed in mouse primary cortical neurons using immunoblot, immunocytochemistry, and cell viability assay. Bromodeoxyuridine (BrdU), an analog of thymidine that can be incorporated into newly synthesized DNA, was intraperitoneally administrated to the AR-97Q mice to assess the levels of DNA synthesis in spinal motor neurons.

Results: Immunohistochemical analysis demonstrated that the spinal motor neurons of AR-97Q mice showed decreased expression levels of TGF-beta-regulated genes, P15 and P21, intracellular accumulation of cell cycle regulators, such as cyclines, E2F1, and PCNA, and hyper-phosphorylation of pRb protein. These phenomena were also observed in autopsy specimens of SBMA patients, but not in non-affected neurons in mice or patients. The uptake of BrdU was increased in spinal motor neurons, but not in Purkinje cells, of AR-97Q mice. TGF-beta inhibitor SD-208 increased the expression levels of cyclines, and intensified the phosphorylation of pRb in primary cortical neurons. These effects are blocked by cycline-dependent kinase inhibitors that suppress cell cycle. Intraventrical administration of SD-208 also increased the expression levels of cycline D1 and induced hyper-phosphorylation of pRb in neurons of the brainstem of AR-97Q mice.

Discussion and conclusion: The present study showed that the inhibition of TGF-beta signaling up-regulates the expression of cell cycle regulators in neurons. The affected motor neurons in mice and patients of SBMA showed increased levels of the proteins that accelerate cell cycle. These findings suggest that cell cycle re-entry is associated with the pathogenesis of neurodegeneration in SBMA.

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P93 INVESTIGATING THE ROLE OF CHRONIC AND ADAPTIVE ER STRESS IN SELECTIVE MOTOR NEURON VULNERABILITY USING AN ALS GENETIC RISK FACTOR AS A MODEL SYSTEM

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Keywords: selective neuron vulnerability, endoplasmic reticulum stress, HFE H63D

Background: Accumulating evidence suggests endoplasmic reticulum (ER) stress as an early cause of motor neuron degeneration in ALS. In the presence of chronic ER stress, the cell can adapt and cell survival is favored or the stress may promote apoptosis. In ALS and other neurodegenerative disorders, persistent ER stress can be tolerated for a long time but eventually it will lead to cell death. It is not known how an adaptive response transforms to apoptosis. The HFE gene encodes an iron regulating protein, and the H63D variant of this gene is found in increased frequency in patients with ALS and may increase the risk of ALS four-fold. We have reported that HFE H63D is associated with chronic but non-lethal ER stress.

Objective: To understand how an ALS genetic risk factor HFE H63D increases the susceptibility to the disease through chronic ER stress. We are testing the hypothesis that predisposing chronic ER stress induced by HFE H63D lowers the stress threshold in motor neurons and promotes selective neuronal vulnerability.

Methods: By chronic exposure to low doses of chemical ER stressors, we recapitulated the adaptive ER stress status in a neuronal cell line and in primary neuronal cultures prepared from wild type and HFE H67D (the mouse equivalent of human H63D) knock-in mouse spinal cord. Serum deprivation was applied as an additional insult and the effect on the cells harboring predisposing adaptive ER stress was determined. The measurement included cell death, apoptosis activation and ER stress levels.

Results: We demonstrated that although treatment with mild ER stressor or serum deprivation alone did not reduce cell survival, combining these two stressors resulted in robust caspase activation and massive cell death. Examination of

major ER stress markers revealed that an ER-stress-induced apoptosis occurred under this dual-stress insult, at least partially due to an increased level of ER stress. These results indicated that a chronic and adaptive ER stress response can be transformed to apoptosis by non-ER-stress insults from the environment. Primary neuronal cells prepared from HFE H67D knock-in mouse spinal cord were more vulnerable to serum deprivation or additional ER stress than the wild type counterparts.

Discussion and conclusions: When adaptive ER stress is challenged neuronal cells are more vulnerable to non-ER-stress-inducing insults. Transformation of predisposing chronic ER stress lowers the stress threshold in neurons which may explain selective neuronal vulnerability and indentifies the HFE mutation as an agent for lowering the stress threshold that promotes neurodegeneration. This has potential therapeutic implications for patients with ALS who harbor HFE H63D mutations.

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P94 UBIQUILIN2-IMMUNOREACTIVE INCLUSIONS REPRESENT A CONVERGENT PATHOLOGY IN A WIDE SPECTRUM OF NEURODEGENERATIVE DISEASES

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Keywords: ubiquilin2, neurodegenerative disease, pathology

Background: Mutations in UBQLN2 have been linked to amyotrophic lateral sclerosis (ALS) and ALS with dementia. The distribution of ubiquilin2 inclusions in ALS and ALS/dementia cases correlates with the clinical symptoms, suggesting a role of ubiquilin2 inclusions in the degenerative process of motor and hippocampal neurons. Mutations in UBQLN2 have been shown to impair the function of ubiquitinated protein degradation, therefore, linking a specific molecular defect in protein degradation to degeneration of motor and hippocampal neurons. The involvement of ubiquilin2 in other neurodegenerative diseases remains to be investigated.

Objectives: This study aims to explore if ubiquilin2 is involved in other neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, frontotemporal lobar dementia that are the top three prevalent neurodegenerative disorders.

Methods: Immunohistochemistry and confocal microscopy were performed on postmortem brains from 63 patients with clinically distinct types of neurodegenerative disease. Brain sections from the 5XFAD transgenic mouse model of Alzheimer disease were also analyzed. Antibodies included those to ubiquilin2, ubiquitin, p62, FUS, TDP43, tau, Aβ and α-synuclein.

Results: Ubiquilin2-immunoreactive inclusions were observed in the affected regions in all of these types of disease. The ubiquilin2-containing inclusions were also immunoreactive with antibodies to ubiquitin and p62, two other critical proteins involved in protein degradation.

Discussion: Our data demonstrate that ubiquilin2 inclusions are a common pathological hallmark in a wide spectrum of neurodegenerative diseases and provide evidence that defects in ubiquitinated protein clearance underlie the pathogenesis in most of the neurodegenerative diseases, indicating a convergent pathway that may be exploited for therapeutic intervention.

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P95 PATHOLOGY ASSOCIATED WITH A NOVEL UBQLN2 MUTATION IN FAMILIAL ALS

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Keywords: TDP-43, UBQLN2, ubiquitin-proteasome system

Background: The disease mechanism underlying amyotrophic lateral sclerosis (ALS) is poorly understood. Gene mutations are the only known cause of ALS. Mutations in SOD1, TDP-43 and FUS are present in approximately 25% of familial ALS cases. Expansions of a hexanucleotide repeat in C9ORF72 have been reported in 39.3% of familial and 7% of sporadic ALS cases (1). Recently, mutations in UBQLN2 (encoding ubiquilin-2) have been identified in approximately 2% of familial ALS (2). A pathological hallmark of ALS is ubiquitin-positive inclusions in degenerating motor neurons. The components of these inclusions variably include TDP-43, FUS, p62 and ubiquilin-2, implicating pathogenic roles for these proteins.

Objectives: To identify ubiquilin-2 pathology in post-mortem ALS spinal cord and fibroblasts.

Methods: Immunofluorescent staining in ALS tissues was performed using anti-ubiquilin-2, anti-TDP-43 or anti-ubiquitin antibodies. Control and patient fibroblasts with ubiquilin-2 mutation were treated with 5 μM proteasome inhibitor MG-132 for 24 hr or 0.5 mM oxidative stress inducer sodium arsenite for 3 hr, followed by immunofluorescent staining using anti-ubiquilin-2 and anti-TDP-43 antibodies. Cells were visualised with confocal microscopy. Toxicity was measured by MTT assay.

Results: In a patient with UBQLN2 p.T487I mutation, ubiquilin-2 co-localised with ubiquitin, TDP-43 and FUS in spinal motor neuron inclusions. The spinal motor neurons of a patient with FUS p.R521C mutation showed ubiquilin-2 co-localisation with FUS and ubiquitin. In fibroblast cells, no ALS-like cellular pathology was found under normal conditions. Inhibition of the ubiquitin-proteasome pathway by MG-132 led to cytoplasmic inclusions in both patient and control fibroblasts. Patient fibroblasts with UBQLN2 p.T487I mutation showed significantly more cytoplasmic inclusions that were positive for both ubiquilin-2 and TDP-43. In contrast, oxidative stress (sodium arsenite) induced TDP-43 positive inclusions that were negative for ubiquilin-2. No differences in toxicity were observed between control and patient fibroblasts following either treatment.

Discussion: Ubiquilin-2 positive inclusions have been found in various types of ALS. We found that ubiquilin-2 pathology was also present in a patient with a novel ubiqui-

lin-2 mutation and a patient with a FUS mutation. Ubiquilin-2 is a member of ubiquitin-like protein family. Mutations in UBQLN2 have been shown to disrupt proteasomal degradation (2). We found that treatment with a proteasome inhibitor, but not an oxidative stress inducer, led to recruitment of inclusions that are positive for both ubiquilin-2 and TDP-43. This suggests that ubiquilin-2 pathology is a consequence of ubiquitin-proteasome pathway disruption. Proteasome inhibition did not alter measured toxicity despite inducing the formation of inclusions.

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P96 RBM45 PATHOLOGY IN SPORADIC AND C9ORF72-LINKED AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: RNA binding protein, stress granules, intracellular inclusion

Background: RNA binding protein pathology is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Using liquid chromatography tandem mass spectrometry (LC/MS-MS), we identified the RNA binding protein RBM45 as a putative ALS biomarker. Immunohistochemistry revealed the presence of RBM45-positive inclusions, similar in appearance to those containing TDP-43, in ALS patients. These could be detected in ALS cases with the C9ORF72 hexanucleotide repeat expansion. Recent demonstrations of unique patterns of p62 and ubiquilin 2 pathology in C9ORF72-linked ALS cases suggest that this form of the disease has a distinct pathological profile.

Objectives: This study was conducted to further describe the prevalence and characteristics of RBM45 pathology in the central nervous system of sporadic and C9ORF72-linked ALS cases, as well as to assess the normal distribution, expression, and subcellular localization of the protein.

Methods: ALS, C9ORF72-linked ALS, FTLD, and age-matched control patient tissues were obtained from the University of Pittsburgh ALS Tissue Bank. Regions examined included spinal cord, hippocampus, cerebellum, and frontal cortex. RBM45 expression was evaluated by immunohistochemistry and immunoblot. Immunofluorescence was used to assess the colocalization of RBM45 with TDP-43, stress granule markers, ubiquitin, p62, and SC35.

Results: Immunoblot results demonstrated the presence of RBM45 in the CSF of ALS and control patients, with modest increases in ALS cases, consistent with our LC-MS/MS data. Using immunohistochemistry we detected RBM45 inclusions in the spinal cord of both sporadic and C9ORF72-linked ALS cases. The number of surviving motor neurons and percentage of motor neurons harboring RBM45 inclusions was higher in C9ORF72-linked ALS cases than in sporadic ALS cases. Inclusions were also found in glia. Cytoplasmic RBM45 inclusions could be seen in the dentate gyrus of FTLD patients and, more rarely, sporadic ALS cases. No inclusions were detected in control cases. RBM45 inclusions were frequently positive for TDP-43, ubiquitin, and stress granule markers. RBM45 also commonly exhibited a speckled nuclear staining pattern that was negative for the nuclear speckle marker SC35.

Discussion: In this study we have further characterized RBM45 pathology in ALS. Moreover, we have added to existing evidence suggesting that sporadic ALS and C9ORF72-linked ALS differ pathologically. Nevertheless, the colocalization of RBM45 inclusions with TDP-43 and stress granule markers suggests common mechanisms of inclusion formation. The speckled nuclear staining pattern observed for RBM45 distinguishes it from TDP-43. This pattern was also separate from SC35-positive nuclear speckles, implying that RBM45 is a component of a distinct subnuclear structure.

Conclusions: We have characterized the expression, distribution, and subcellular localization of RBM45 and RBM45 inclusions in ALS and control subjects. Ongoing studies will determine RBM45's role in RNA metabolism, stress response, and how these processes might contribute to neurodegeneration.

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P97 NUCLEAR RNA FOCI IN HEXANUCLEOTIDE EXPANDED C9FTD/ALS

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Keywords: RNA, foci, hexanucleotide

Background: Nuclear RNA foci of the expanded hexanucleotide repeat were reported as a feature of c9FTD/ALS in the landmark DeJesus-Hernandez *et al.* paper of 2011. This finding is fundamental in addressing whether disease biology is mechanistically mediated through haploinsufficiency or RNA toxicity. As yet, this critical finding has not been reproduced.

Objectives: To seek and characterize nuclear RNA foci in c9FTD/ALS.

Methods: Custom fluorescent locked nucleic acid (LNA™) probes (Exiqon) were designed against the GGGGCC expansion and a control sequence. Probes were hybridized using a customized protocol, and examined at 100X magnification on a fluorescent confocal microscope. Specificity of probe binding was confirmed by competition with non-fluorescent probe and by RNase-treated controls. FFPE tissue sections from confirmed C9ORF72 repeat expansion nervous systems (n = 3) were tested at multiple CNS levels, sALS (n = 5), and non-neurologic controls (n = 5) were tested in parallel

In addition, we studied multiple primary fibroblast and immortalized lymphoblast cell lines.

Results: We found nuclear RNA foci exclusively in the c9FTD/ALS tissues. The foci are dumbell shaped and uniform in size, with fluorescent signals measuring approximately 0.5 um across and 0.2 um wide. The foci were seen in about 20–50% of the motor neuron nuclei contained in a histological cross-section (6um) of lumbar spinal cord. The usual count was 1 focus per nucleus, but occasionally 2 or more foci were observed (up to a maximum of 12). Foci were found in the all three spinal levels, the motor cortex and cerebellum. They were observed in both neurons and glial cells. In addition, they were seen in up to ~30% of cultured primary fibroblasts and immortalized lymphoblast cells, where many foci (> 10) were commonly noted within a single nucleus.

Discussion and conclusions: We confirm that nuclear foci of hexanucleotide RNA repeats are a hallmark feature of c9FTD/ALS. This supports the hypothesis that the expanded repeats exert their toxicity at the RNA level. Additionally, we validated two non-neuronal cell types which can be easily manipulated in a laboratory setting as containing this RNA feature. Since the RNA foci appear in multiple cell lines, including non-neural cells, their presence alone does not appear to explain select neuronal vulnerability.

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P98 GAMMA-SYNUCLEIN PATHOLOGY IN ALS

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Keywords: axonopathy, protein aggregation, phagocyting glial cells

Background: γ-synuclein, a small, cytosolic, aggregation prone protein expressed by select populations of neurons is not currently considered a common contributor to neurological disease. We have previously demonstrated that mice expressing high pan-neuronal levels of γ-synuclein progressively accumulate the protein throughout the CNS, accompanied by the selective degeneration of motor neurons and their axons, in a pathomorphological pattern resembling that typical of ALS mouse models.

Objectives: Due to the auspicious combination of γ-synucleins normal presence in motor neurons, high aggregation propensity and the ALS-like phenotype in mice overexpressing the protein, we aimed to explore the possibility that it might play a role in human motor neuron disease pathogenesis.

Methods: Human tissue samples and detailed pathology reports were obtained from the MRC London Neurodegenerative Diseases Brain Bank: sALS (n = 16), f SOD1-fALS (n = 3), FUS-fALS (n = 3), Alzheimer's disease (n = 6), Lewy body dementia (n = 1), Parkinson's disease case (n = 1) and control cases (n = 8). Spinal cord sections were immunostained with two separate antibodies specific to human γ-synuclein. A set of antibodies against markers of specific cell types were used for co-localisation studies. Sequential protein extraction was also carried out upon several samples.

Results: Immunohistochemical screening revealed a subset of sporadic (8/16) and familial (2/6) ALS cases with a novel pathology characterized by the accumulation of γ-synuclein within the dorsolateral corticospinal tracts, associated with all stages of upper motor neuron atrophy. A portion of these structures was found in association with phagocytic glial cells positive for HLA-DRα and Mac-2/Galectin-3. Sequential fractionation of proteins from spinal cord tissues revealed detergent-insoluble γ-synuclein species specifically in the dorsolateral corticospinal tracts of ALS patients with γ-synuclein-positive profiles in this region.

Discussion: We show a novel type of pathological profile in the descending motor columns of approximately half of ALS patients included in this study, revealed by immunostaining with antibodies specific to γ-synuclein, but not with antibodies to other proteins typically associated with ALS pathology. The specific presence of detergent insoluble γ-synuclein species in the extracts from the profile-positive dorsolateral column but not from the profile-negative anterior horn suggests these structures contain aggregated γ-synuclein. Interestingly, a portion of these structures was associated with Mac-2/galectin-3 positive phagocytic glia, a novel class of which were recently associated with γ-synucleins dependent neurodegeneration of the optic nerve in a mouse model of glaucoma (Nguyen et al., 2011), suggesting the a role for the protein in a common mechanism of axonal degeneration.

Conclusion: Our observations suggest that in some cases the pathological aggregation of γ-synuclein might contribute to the pathogenesis of ALS.

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P99 H63D HFE MUTATION MAY CONTRIBUTE TO ALS PATHOGENESIS VIA MITOCHONDRIAL PATHWAYS

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Keywords: mitochondria, H63D HFE, SOD1

Background: The H63D polymorphism in the HFE gene, which regulates iron metabolism, is present in about 30% of sporadic ALS cases. *In vitro* research suggests H63D HFE causes mitochondrial dysfunction, a process separately implicated in ALS pathogenesis. Investigating the effects of H63D polymorphism in ALS patient muscle biopsies, as well as relevant cell culture and mouse models, in the context of mitochondrial processes may provide insights into ALS pathophysiology.

Objectives: To determine if H63D polymorphism contributes to ALS etiology by measuring: 1) levels of soluble superoxide dismutase (SOD1) protein in muscle biopsies from ALS patients with H63D HFE; 2) levels of mitochondrial electron transport chain (ETC) proteins in human neuroblastoma cells stably transfected with H63D HFE; and 3) ETC protein levels in transgenic mice with H67D HFE (analogous to human H63D HFE).

Methods: Muscle biopsy samples which had previously been obtained from ALS patients with definite, probable, probable laboratory-supported, or possible ALS were separated into two groups: wild type (WT) HFE or H63D HFE (heterozygous or homozygous). Immunoassay was used to

determine levels of soluble SOD1 protein. SH-SY5Y human neuroblastoma cell lines stably transfected with empty vector, WT HFE or H63D HFE were collected, and mitochondria were isolated using differential centrifugation. Mitochondrial fractions were analyzed for protein levels of complex IV using Western blot. Quadriceps muscle tissue from 6- month-old WT or transgenic mice hetero- or homozygous for H67D mutation was collected, mitochondria were isolated using differential centrifugation, and mitochondrial complex IV levels were analyzed using Western blot. The study of all human samples was approved by our IRB.

Results: Muscle biopsy results demonstrated that H63D HFE ALS patients ($n = 16$) had 40.7% lower expression of soluble SOD1 protein versus ALS patients with WT HFE ($n = 22$, $p < 0.005$). *In vitro* results demonstrated that neuroblastoma cells stably transfected with H63D HFE had 61.7% lower expression of mitochondrial complex IV protein versus cells transfected with WT HFE ($p < 0.05$). Mice either hetero- ($n = 3$) or homozygous ($n = 6$) for H67D HFE had approximately 20% lower expression of mitochondrial complex IV protein versus WT mice ($n = 6$) ($p < 0.01$ for both comparisons).

Discussion and conclusions: Because previous research suggests expression of soluble SOD1 is lowered in lymphocytes and affected CNS regions in sporadic ALS, the finding that H63D HFE polymorphism appears to decrease levels of soluble SOD1 in muscle is clinically relevant. H63D HFE also appears to disrupt mitochondrial function, as reflected by lowered expression of complex IV, a terminal ETC complex. One possible mechanism for the observed mitochondrial changes may be accumulation of insoluble SOD1 aggregates targeting mitochondrial processes. Given the role of altered non-mutant SOD1 levels as well as mitochondrial dysfunction in ALS, our study further implicates the H63D polymorphism in ALS pathogenesis.

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P100 mRNA EXPRESSION PATTERN OF AXON GUIDANCE PROTEINS IN HUMAN POST MORTEM MOTOR CORTEX IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: axon guidance proteins, semaphorins, plexins, neuropilins

Background: Several findings support the hypothesis of early subclinical involvement of neuromuscular junctions and terminal axons followed by subsequent disease progression towards motor neuron cell bodies ('dying back'-hypothesis) in ALS. Axon guidance proteins have first been described as steering molecules for developing axons but are also important for maintenance of neuronal structure, axonal transport and synaptic function in the adult nervous system. Aberrant function or expression of axon guidance proteins may therefore contribute to the pathological changes in motor neuron connectivity in ALS.

Objectives: In the present study we assess the distribution and expression levels of the different axon guidance proteins (semaphorin 3A, 3B, 3C, 3D, 3E, 3F) and their receptors (plexin PlxA1, PLxA2, PlxA3, PlxA4 und neuropilin Np1, Np2) in human post mortem motor cortex of ALS patients and age matched controls.

Methods: Quantitative real time PCR (qRT-PCR) and in situ hybridization histochemistry (ISH) were used for analysis of the mRNA expression patterns of different axon guidance proteins and their receptors in human post mortem motor cortex of ALS patients ($n = 5$) and age matched controls ($n = 5$). In ISH experiments, mRNA expression was quantified macroscopically by densitometric analysis of digitized film autoradiograms, the expression at the cellular level was studied by liquid emulsion autoradiography. Corresponding rotein expression patterns were studied by immunohistochemistry.

Results: mRNA expression of the semaphorin receptors plexin A3 and neuropilin 1 was significantly lower in ALS motor cortex as measured by qRT-PCR. These results could be confirmed by ISH and immunohistochemistry. Immunohistochemistry in addition revealed higher expression of the axon guidance protein semaphorin 3A, which is known as an axon growth inhibitor.

Discussion and conclusion: These results suggest increased expression of the axon growth inhibitor semaphorin 3A with possibly reactive reduced expression of the related receptors in ALS tissue. It has already been shown in a rat model of spinal cord injury and an *in vitro* axotomy model that semaphorin inhibitors can promote axonal regeneration. According to our results, they should be further evaluated as novel therapeutic option in ALS.

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P101 CSF TRACE-ELEMENTAL ANALYSIS OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS BY X-RAY MICROFLUORESCENCE WITH SYNCHROTRON RADIATION

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Keywords: cerebrospinal fluid, trace elements, protein aggregates

Background and objectives: Biochemical analysis of the Cerebrospinal Fluid (CSF) has immense potential for diagnosis and prognosis in many neurological diseases (1). CSF can provide a repository of ALS pathological information. Histopathologically, in ALS, there is marked neuronal loss and inclusion bodies containing protein aggregates (2). Abnormal protein-protein interactions remain uncertain and a role for trace elements has been suggested. The aim of this study was to investigate trace element levels of ALS CSF samples.

Methods: ALS (10) and control (6) CSF samples were treated for trace elemental analysis by X-ray microfluorescence with synchrotron radiation. Measurements were

carried out at the XRF beam line at the Synchrotron Light National Laboratory (Campinas, Brazil). The intensity of X-Ray specter for each element was obtained with AXIL software (distributed by International Agency of Atomic Energy). These obtained data were analyzed statistically by General Linear Models, in a multivariate based method.

Results: In this study, the following elements were analyzed: aluminium, bromine, calcium, chlorine, copper, chromium, iron, potassium, phosphorus, nickel, rubidium, silicon, sulfur and zinc. In ALS CSF samples we observed significant increased concentration of calcium, chlorine and potassium when compared to control samples.

Discussion: Due to its close proximity to the central nervous system parenchyma, CSF is likely to be used for detecting dynamic information about pathological processes of the brain. Excitotoxicity is one of many factors implicated in ALS pathogenic process. The intracellular calcium influx seems to contribute to neurodegeneration in multiple pathways, conducing cell to death (3). Activation of potassium and chlorine channels by increased calcium level was already described and prolonged efflux of both could be involved in apoptosis (4). The elevated levels of these three elements in CSF can reflect an abnormal activation of potassium and chlorine channels, indicating a possible molecular pathway involved in ALS pathogenic process.

Conclusion: Analysis of biological samples provides a powerful strategy for investigating pathological processes. Moreover, extensive studies indicated an important role of trace elements in ALS disease and our data are in agreement with these. The role of trace elements in ALS pathogenesis must be more investigated since the identification of altered elements can be a significant step in ALS research and future therapy.

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THEME 5 GENETICS

P102 HAS THE TIME NOW COME FOR A REVISION OF THE AMYOTROPHIC LATERAL SCLEROSIS GENE CLASSIFICATION SYSTEM?

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Keywords: ALS genetic classification, ALS phenotypes, familial ALS

Background: The past twenty years have seen great advances in identifying genes causing Amyotrophic Lateral Sclerosis (ALS) and a numbered gene classification system currently exists consisting of fourteen genes (1–5). However this classification system does not accurately reflect the clinical syndrome of ALS and there are several anomalies. For example, genes for very slowly progressive motor syndromes, for juvenile onset, or for pure upper motor neuron syndromes are over-represented and listed as ALS genes. On the other hand, genes known to be responsible for a significant proportion of ALS are listed as ALS-FTD (Frontotemporal Dementia) genes. We therefore propose an overhaul of the existing ALS genetic classification system.

Objectives: The aim was to design a rational ALS genetic classification system.

Methods: Clinical syndromes of genetic ALS were compared with criteria for classical adult-onset ALS. Genes known to cause ALS as a phenotype were reviewed for classification as ALS genes.

Results: Of the fourteen current numbered ALS genetic syndromes (ALS 1 - ALS 13 and ALS-X), nine are consistent with classical adult-onset ALS. These genetic syndromes with their respective gene names in parentheses are 1. ALS 1 (SOD1), 2. ALS 6 (FUS), 3. ALS 8 (VAPB), 4. ALS 9 (ANG), 5. ALS 10 (TARDBP), 6. ALS 11 (FIG4), 7. ALS 12 (OPTN), 8. ALS 13 (ATXN2) and 9. ALS-X (UBQLN2). A further two syndromes, ALS-FTD (C9orf72) and ALS-IBM (VCP), which are not numbered within the current system could be included in a new system.

Discussion: Based on the evidence reviewed we make the case that only nine of the fourteen genetic syndromes currently numbered in the ALS gene classification system should be included in a new proposed system. We would include a further two unnumbered syndromes making a total of eleven genetic syndromes causing classical adult-onset ALS.

Conclusion: The current classification system is derived from genetic studies which are driven by historically available genetic techniques rather than the clinical syndromes seen in ALS clinics. A rational redesign would benefit clinicians, patients and researchers.

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P103 CLASSIFICATION OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS BY FAMILY HISTORY: EFFECTS ON FREQUENCY OF GENES MUTATION

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Keywords: familial ALS, genetics

Background: Though the distinction between familial and sporadic ALS is widely accepted, the definition of FALS has not been clearly established. Recently, criteria for FALS were proposed, incorporating family history and genetic analysis. The aim of our work was to classify FALS on the base of proposed criteria and to determine whether frequency of mutations in major ALS genes varies in different FALS categories.

Methods: Included in the study are 53 FALS families. Patients with at least two first- or second-degree affected relatives were classified as definite FALS. Patients with only one affected relative were classified into two categories: probable FALS, when a first- or second-degree relative was affected, and possible FALS, when a distant relative had the disease. Seven ALS-associated genes, including *SOD1, TARDBP, FUS, ANG*, *ATXN2*, *OPTN* and *C9ORF72*, were analyzed.

Results: Thirteen patients (24.5%) were included in the definite group. The great majority of our FALS cases (40/53, 75.5%) were families with only two affected relatives; of these, thirty-one (58.5%) were included in the probable and nine (17%) in the possible FALS category, respectively. The percentage of mutations was 61.5% in definite, 41.9% in probable and 11.1% in possible FALS. With respect to probable FALS, if cases with parent to child transmission of the

disease were considered separately, the mutational load increased to 61.5%, as observed in definite FALS.

Conclusions: Our findings provide evidence that frequency of mutations in currently known ALS genes varies widely among different FALS categories. Families with only two affected relatives have heterogeneous genetic components, the chance to detect mutations being higher in cases with parent to child transmission.

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P104 GENETICS OF ALS IN ITALY: A POPULATION-BASED STUDY

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Keywords: population based study, SOD1, TARDBP, ANG, FUS, OPTN, C9ORF72

Background: According to a recent systematic review, 5% of amyotrophic lateral sclerosis (ALS) patients have a positive family history for the disease. No studies have systematically assessed the frequency of mutations of ALS-related genes in a population-based setting.

Aim: To assess the frequency and clinical characteristics of patients with mutations of major ALS genes in a prospectively ascertained, population-based epidemiological series of cases.

Methods: The study population includes all ALS cases diagnosed in Piemonte, Italy, from January 2007 to June 2011. Mutations of *SOD1, TARDBP, AN7 7G, FUS, OPTN* and *C9ORF72* have been assessed.

Results: Out of the 475 patients included in the study, 51 (10.7%) carried a mutation of an ALS-related gene (*C9ORF72*, 32; *SOD1*, 10; *TARDBP* 7; *FUS*, 1; *OPTN* 1; *ANG* none). A positive family history for ALS or frontotemporal dementia (FTD) was found in 46 (9.7%) patients. Thirty-one (67.4%) of the 46 familial cases and 20 (4.7%) of the 429 sporadic cases had a genetic mutation. According to logistic regression modeling, besides a positive family history for ALS or FTD, the chance to carry a genetic mutation was related to the presence of co-morbid FTD (OR 3.5; $p = 0.001$), and age at onset ≤ 54 years (OR 1.79; $p = 0.012$).

Conclusions: We have found that ~11% of ALS patients carry a genetic mutation, with *C9ORF72* being the commonest genetic alteration. Co-morbid FTD or a young age at onset are strong indicators of a possible genetic origin of the disease, and should prompt to send the patient for genetic counseling.

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P105 MUTATIONAL SCREENING OF C9ORF72, SOD1, TARDBP AND FUS GENES IN AN ALS COHORT FROM THE NORTH-EASTERN PART OF ITALY

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Keywords: genetics, ALS genes, epidemiology

Background: Mutations in *C9ORF72*, *SOD1*, *TARDBP* and *FUS* genes appear as the most common genetic cause of familial (FALS) and sporadic (SALS) forms of ALS.

Objectives: To assess the frequency of ALS genes mutations in a large cohort of ALS patients referred to the ALS Clinic of the University of Padova, in the North-Eastern part of Italy.

Methods: We searched for the expanded GGGGCC hexanucleotide repeats in the C9ORF72 gene and we sequenced all coding exons of *SOD1* and *FUS* genes and two exons of *TARDBP* (exon 4 and 6) in 351 ALS patients (30 FALS and 321 SALS).

Results: 10 pathogenic missense mutations were found in 11 unrelated patients: 6 *SOD1* (3 in FALS and 3 in SALS), 3 *TARDBP* (1 in FALS and 3 in SALS) and 1 *FUS* (FALS). All the mutations but one have already been reported: a novel *TARDBP* mutation was identified in exon 4. Screening of 200 chromosomes failed to report the mutation. Expanded repeats in *C9ORF72* were found in 21 patients (10 FALS and 11 in SALS). From a clinical point of view, we could observe a shorter disease lifespan and an increased dementia comorbidity in carriers of the expanded hexanucleotide repeats in *C9ORF72*.

Conclusions: While a new mutation was found in *TARDBP* (the second reported so far in exon 4), we could observe a lower frequency of TARDBP and FUS mutations in our ALS cohort. SOD1 and C9ORF72 screening confirmed results reported in other series, thus making the two genes as the most common genes involved in ALS also in this part of Italy.

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P106 C9ORF72 REPEAT EXPANSION IN ITALIAN ALS PATIENTS: EVIDENCE OF A FOUNDER EFFECT

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Keywords: C9ORF72, genetics

Background: A hexanucleotide repeat expansion (RE) in *C9ORF72* gene was recently reported as the main cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In populations of European descent the mutational frequencies ranged from 23–47% in familial ALS (FALS), 4–5% in sporadic ALS (SALS), 12–29% in FTD and 6–86% in ALS-FTD patients.

Objectives: the aim of our study was to screen *C9ORF72* in a large cohort of FALS and SALS patients of Italian origin, with or without frontotemporal dementia.

Methods: We screened *C9ORF72* in a large cohort of 259 FALS, 1275 sporadic SALS and 862 healthy control individuals of Italian descent. RE in *C9ORF72* was analyzed by repeat-primed PCR followed by fragment lenght analysis using the GeneScan software (Applied Biosystems). For haplotype analysis we utilised genome-wide association (GWA) data from SALS and control cohorts previously genotyped on Human660W-Quad BeadChip (Illumina).

Results: We found RE in 23.9% FALS, 5.1% SALS and 0.2% controls. Two cases carried the RE together with mutations in other ALS-associated genes. The phenotype of RE carriers was characterized by bulbar-onset, shorter survival and association with cognitive and behavioural impairment. Extrapyramidal and cerebellar signs were also observed in few patients. Genotype data revealed that 95% of RE carriers shared a restricted 10-SNP haplotype within the previously reported 20-SNP risk haplotype, detectable in only 27% of non-expanded ALS cases and in 28% of controls, suggesting a common founder with cohorts of North European ancestry.

Discussion and conclusions: our data confirm that RE in *C9ORF72* represents the main genetic cause of both FALS and SALS also in the Italian population. However, the identification of RE both in controls and in patients with additional pathogenic mutations suggests that penetrance and phenotypic expression of *C9ORF72* RE may depend on additional genetic risk factors.

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P107 UBIQUILIN NEUROPATHOLOGY AND FREQUENCY OF C9ORF72 HEXANUCLEOTIDE EXPANSION IN A LARGE COHORT OF ALS AND ALS/FTD PATIENTS

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Keywords: C9ORF72, ubiquilin, pathology

Background: C9ORF72-hexanucleotide repeat $(GGGGCC)^n$ was found to be expanded in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTD). Incidence of expansion was reported in relative small sample sizes, varying from 22.5% to 46.4% in FALS, 4.1% to 21% in SALS, and 10.4% to 50.6% in FALS/FTD. The pathogenic mechanism of the C9ORF72-linked ALS/FTD remains largely unknown.

Objectives: To evaluate the frequency of C9ORF72-hexanucleotide expansion in a large cohort of ALS and ALS/FTD of North America patients and to characterize the C9ORF72-related neuropathology.

Methods: A cohort of 1,807 cases without known gene mutations, including 420 FALS, 1235 SALS, 134 FALS/FTD, 18 SALS/FTD was analyzed. In addition, a total of 704 control subjection was also included. Expansion of C9ORF72-hexanucleotide was determined by repeat-primed PCR and further confirmed by Sanger sequencing in 281 cases. Samples with large number of repeats were also analyzed by Southern blot. Immunohistochemistry and confocal microscopy were performed on hippocampal sections from patients with C9ORF72 mutations.

Results: The frequencies of C9ORF72-hexanucleotide expansion were 43.8% in FALS, 4.5% in SALS, 61.2% in FALS/FTD, and 5.6% in SALS/FTD, respectively. The expansion frequency in the whole FALS population was 29.1%. No expansion was found in the 704 controls. The allele (GGGGCC)2 represented the most frequent allele in the control cohort, which accounted for 50.4% of the total alleles. Rare alleles with limited repeat expansion were observed in controls ((GGGGCC)21, n = 1; (GGGGCC)20, n = 3)), but no repeat expansion over 21 was observed. The ubiquilin2-positive aggregates in the molecular layer of the dentate gyrus and CA1-CA4, which were also ubiquitin- and p62-positive, but TDP43- and FUS-negative, appeared to be a characteristic pathology in the C9ORF72-linked cases.

Discussion: The hexanucleotide expansions in the intron of C9ORF72 represent the most frequent mutations identified to date in familial ALS and ALS/FTD cases. The presence of the ubiquilin2-positive aggregates in the molecular layer of the dentate gyrus and CA1-CA4, which were ubiquitin- and p62-positive, but TDP43- and FUS-negative, suggests that ubiquilinopathy is a specific and common pathological

hallmark in both ALS and ALS/FTD. Our data indicate that protein/organelle degradation defect may be a common mechanism underlying ALS and FTD.

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P108 C9ORF72 HEXANUCLEOTIDE EXPANSION IS ASSOCIATED WITH AGGRESSIVE DISEASE PROGRESSION AND FRONTOTEMPORAL DEMENTIA COMORBIDITY IN PATIENTS WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: C9ORF72, frontotemporal dementia, comorbidity

Background: Recently, GGGGCC hexanucleotide repeat expansions in the gene *C9ORF72* have been identified as a novel cause of familial amyotrophic lateral sclerosis (fALS) and frontotemporal dementia (FTD) cases of European ancestry. Yet, little is known concerning the impact of an expansion on clinical characteristics in these patients.

Objective: The aim of this study was to compare detailed clinical characteristics of patients compatible with carrying a *C9ORF72* hexanucleotide expansion as determined by PCR analysis with those of patients with no known or other gene mutations in a total of 109 fALS index patients. Additionally, we aimed to investigate whether *C9ORF72* patients show a higher comorbidity with FTD then fALS patients with no known or other gene mutations.

Methods: We studied a cohort of 109 fALS index patients. For all patients, detailed clinical and demographic data were obtained by interviewing patients and/or relatives and by going through medical records. In 38 patients (6 with hexanucleotide expansion, 32 controls), we checked whether FTD was also diagnosed concomitant to ALS. We used a repeat-primed polymerase chain reaction (PCR) assay to detect GGGGCC hexanucleotide repeat expansions in the gene *C9ORF72*.

Results*:* PCR based analyses were compatible with a hexanucleotide expansion in 26 cases (23.9 %). Mean disease duration from symptom onset in these patients was substantially shorter than in patients with no known or other gene mutations. In addition, patients in the *C9ORF72*-group had a significantly higher proportion of bulbar onsets compared to the fALS control group. In 2 patients, FTD was diagnosed concomitant to fALS. These patients both belonged to the *C9ORF72*-group. Thus, 1/3 of the *C9ORF72*-patients that were checked for this comorbidity were diagnosed with FTD. No significant gender differences between the two groups could be observed.

Discussion: The molecular results presented here show that *C9ORF72* hexanucleotide expansions may account for about 1/5 of this large German cohort of fALS cases. *C9ORF72* hexanucleotide expansions seem to be associated with a significant proportion of FTD concomitant to fALS in these patients. Our data substantiates the notion that ALS and FTD share similar pathogenic and genetic mechanisms and may be in fact both part of the same disease spectrum. Furthermore, we show that patients with molecular analyses compatible with a hexanucleotide expansion in *C9ORF72* seem to show a more aggressive disease course and significantly shorter disease duration as well as a higher frequency of bulbar onset compared to patients with no known or other gene mutations.

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P109 C9ORF72 HEXANUCLEOTIDE REPEAT STUDY IN FAMILIAL AND SPORADIC ALS SPANIARDS PATIENTS

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Keywords: C9ORF72, sporadic ALS, familial ALS

Background: The recent discovery of the genotype-phenotype relation between ALS and a hexanucleotide expansion in the C9ORF72 gene is the most important clue in the history of this pathology. In the present study, we try to bring more data about the clinical and molecular characteristics of the molecular spectrum of the hexanucleotide repeat expansion in *C9ORF72*. And furthermore, we try to investigate in the families of previously considered sporadic ALS in order to clarify how we should give genetic counseling in the future, and which variables we should take into account to integrate in the clinical management of ALS patients.

Methods: We have studied a total of 850 samples from ALS patients both sporadic and familial. The repeat-primed polymerase chain reaction method was used to identify the GGGGCC hexanucleotide expansion in the C9ORF72 gene, as previously described.

Results: The hexanucleotide repeat expansion in C9ORF72 gene (defined as more than 30 repeats) was found in 54 out of 700 ALS cases, thus representing 6.4% of our ALS Spaniard Caucasian cohort. Pathological expansions accounted for 27.1% of fALS and 3.2% of sALS individuals.

Conclusion: Our results conclude that all the phenotypes positive for the hexanucleotide expansion in *C9ORF72* gene are actually familial cases, but in the case of sporadic ones, they are hidden because of a lack of information about the family members.

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P111 DIFFERENT CHARACTERISTICS OF GGGGCC-HEXANUCLEOTIDE REPEATS EXPANSION IN C9ORF72 IN JAPANESE, TURKISH, SWISS AND SWEDISH POPULATIONS

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Keywords: C9ORF72, hexanucleotide repeat expansion, repeat primed-PCR

Background: The expanded GGGGCC-hexanucleotide repeats in the C9ORF72 were recently identified as a cause of ALS and FTD. In selected patient materials, 23–46% of the ALS patients with family histories and 5–21% of the ALS patients without family histories were affected by the pathological hexanucleotide repeat expansion in C9ORF72.

Objectives: To determine the normal hexanucleotide repeat numbers in C9ORF72 in four large control groups with different ethnic background.

Methods: Total 1285 normal control cases: 647 Swedish and 239 Swiss for the European, 199 Turkish for the Middle Eastern, and 200 Japanese for the Far East Asian. Genomic DNA was screened using the repeat primed polymerase chain reaction (RP-PCR) method. Fragment length analysis was performed on Applied Biosystems 3730 genetic analyzer and visualized using Peak Scanner Software. We classified the RP-PCR results into four types: A was normal type, B was pathological long expansion with a "sawtooth pattern", C had intermediate expansion, and Q was unclassifiable.

Results: The average age of the Swedish cases was 59.3 ± 14.0 (mean ± SD) years old, and the Japanese cases was 51.8 ± 8.6 years old. The male and female ratio was 1.06 in Swedish, 1.52 in Swiss, 0.99 in Turkish, and 1.63 in Japanese. The B type (pathological long expansion) was found in the only European ethnic: 3 in Swedish and 1 in Swiss, but could not be found in the other populations. The C type (middle expansion) was found in 38 Swedish (5.9%), 14 Swiss (5.9%), and 4 Turkish (2.0%) individuals, but was not found in the Japanese group.

Discussion and conclusions: According to the previous studies, the pathological hexanucleotide repeat expansion was found 0–0.57% of the individuals in control groups. Based on this control case study it is present in 0.45% in the European group and 0% in Turkish and Japanese groups. And the middle expansion type was also found at a higher percentage in the European group, suggesting that the repeat numbers of the hexanucleotide in C9ORF72 is different depending on the ethnic origin of the population.

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P112 SOD1, ANG, TARDBP MUTATIONS AND ATXN2 TRINUCLEOTIDE REPEAT VARIATION IN ALS PATIENTS FROM SERBIA

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Keywords: mutations, SOD1, ANG, TARDBP, ATXN2, founder

Background: *SOD*, *ANG* and *TARDBP* gene mutations , as well as higher presence of longer normal (CAG) alleles of *ATAXIN 2* gene, have been identified in amyotrophic lateral sclerosis (ALS).

Objectives: The aim of the study was to determine the the frequency of *SOD1, ANG, TARDBP* gene mutations and distribution of *ATXN2* alleles in Serbian familial and sporadic ALS patients.

Methods: The population comprised 191 patients, of which 37 were familial, with probable or definite ALS diagnosed at the Clinic of Neurology, Clinical Center of Serbia. Samples were collected also from 100 healthy individuals, sex and age matched to patients. All participants signed a written consent for the research. Blood samples were collected over the past 10 years and processed at the Faculty of Biology, University of Belgrade. Coding regions and exon-intron boundaries of *SOD1* (5 exons), *ANG* (exon 1 and 2) and *TARDBP* (5 exons) genes were amplified and analyzed using direct sequencing on ABI 3130 genetic analyzer. Also, the polyglutamine repeat region in the exon 1of the ATXN2 gene was amplified using fluorescent label primers and subjected further to fragment analysis.

Results: We found 6 pathogenic missense (4 SOD1, 1 ANG, 1 TARDBP) and one splicing mutation in 27 (72.97%) FALS and 12 (7.90%) SALS patients, of which *SOD1* gene mutations were the most frequent (70.27% FALS and 7.14% SALS). *SOD1* L144F mutation in exon 5 was identified with high frequency in both, 22(59.45%) FALS and 4 (2.59%) SALS patients, while A145G, also in exon 5, was identified only in 4 (10,81%) FALS patients. Novel P66S mutation of *SOD1* gene exon 3 was registered in one SALS patient as well as complex change (IVS2 + 1G > A + IVS2 + 3 G > A) (p.A55 + 14 STOP) in another. D90A SOD1gene mutation, was observed in 2 SALS patients with homozygous and 3 with heterozygous state. In SALS patients mutation was observed in the *ANG* gene, one p.P-4S along with one „benign" variant p.I46V, while one mutation in *TARDBP* gene (p.M337V) was seen in one FALS patient. Analysis of (CAG)n repeats in the *ATXN2* gene sequence showed the presence of one SCA 2 allele (> 35 CAG) and two alleles in higher normal range (> 30 CAG) only in ALS patiens. Haplotype analysis revealed the common origin for chromosomes bearing the *SOD1*-L144F, *SOD1*-A145G and *SOD1*-D90A, except for one D90A chromosome which common origin with other *D90A* chromosomes was ruled out.

Conclusion: this study confirms previously published studies, identifies new genetic associations with ALS, and provides insight in genotype/phenotype aspects of both previously reported and novel SOD1 gene mutations of which some could be specific to this region.

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P113 DE NOVO P525L FUS MUTATION FOUNDED IN A YOUNG SPORADIC SPANIARD WOMAN WITH ALS

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Keywords: FUS, young ALS, basophilic inclusions

Background: Juvenile amyotrophic lateral sclerosis (ALS) with basophilic inclusions is a form of ALS characterized by protein deposits in motor neurons that are morphologically and distinct from the rest of the considered sporadic ALS.

Methods: We identified neuropathologically 1 patient with juvenile ALS with basophilic inclusions and tested the hypothesis that specific RNA binding protein pathology may define this type of ALS. Immunohistochemical findings prompted us to sequence the fused in sarcoma (*FUS*) gene.

Results: Motor symptoms began at the age of 22. Disease progression was rapid without dementia. No family history was identified. Sequencing of all 15 exons of the *FUS* gene in 3 patients revealed the mutation c.1574C_T (P525L). On postmortem neuropathological examination abundant FUS-positive basophilic inclusions were found in motor cortex, brainstem motor nuclei, subthalamic region and dentate nucleus of the cerebellum.

Conclusion: Juvenile ALS with basophilic inclusions is a FUS proteinopathy. The *FUS* c.1574C_T (P525L) is associated with this phenotype, and it appears in the Spaniard population too.

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P114 MUTATION IN UBIQUILIN4 IN ALS PATIENT AFFECTS THE UBIQUITIN PROTEOSOME SYSTEM AND AUTOPHAGY IN VITRO

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Keywords: ubiquitin proteosome system, autophagy, ubiquilin, ubiquilin4

Background: Growing evidence supports the notion that impairment of autophagy is associated with the formation of protein aggregates and neurodegeneration including ALS. We recently reported mutations in Ubiquilin2, a member of the Ubiquilin family affecting both the Ubiquitin Proteosome System (UPS) and Autophagy *in vitro*. We have now identified a mutation in Ubiquilin4 gene in a familial ALS patient. Ubiquilin4 is a member of the Ubiquilin family and also plays a role in the regulation of proteasomal protein degradation.

Objectives: The aim of the current study is to test whether the identified mutation in Ubiquilin4 affects the UPS and autophagy *in vitro*.

Methods: Genomic DNA from Familial ALS cases as well as controls was subject to sequencing. Recombinant Ubiquilin4 wild type and mutant tagged to mCherry were used to transiently transfect Neuro2A cells (N2A). Confocal microscopy, using Zeiss LSM 510 Meta laser scanning confocal microscope, was performed to evaluate the expression of the Ubiquilin4 wild type and mutant and conduct colocalisation with autophagosome markers and ALS linked proteins. For flow cytometry, N2A were co-transfected with an early autophagosome marker microtubule-associated protein 1A/1B-light chain 3 tagged to GFP (LC3-GFP) and autophagosome maturation marker (Rab7-GFP). Cells were analyzed using a BD LSRFortessa flow cytometer and BD FACSDiva software.

Results: We identified a mutation in the exon 3 of Ubiquilin4 gene that was absent from controls. We also found four single nucleotide polymorphisms (SNPs) that were present in the controls. N2A cells transiently transfected with Ubiquilin4 tagged to mCherry showed punctuate structures in both wild type and mutant. Flow cytometry revealed a significant accumulation of LC3-GFP which corresponds to an inhibition of autophagic activity in mutant Ubiquilin4 transfected cells. With a milder action on the Ubiquitin accumulation. Consistent with this, we observed that both wt and mutant Ubiquilin4 co-localize with LC3, p62 and subsequently Ubiquilin2. However we found a significant decrease in the Rab7-GFP accumulation in the mutant Ubiquilin4 transfected cells which may indicate impairment at the autophagosome late endosome formation and exacerbating the decrease in autophagy activity.

Discussion and conclusion: These data suggest that mutant Ubiquilin4 impairs the UPS and autophagy and may suggest a role in ALS pathogenesis. Such a mechanism could participate in the accumulation of the aggregation-prone proteins in ALS, and affects essential regulatory functions of autophagy and the UPS. Hence, degradation pathways related proteins, like Ubiquilins (Ubiquilin 2 and 4), represent attractive targets for designing rational therapeutics in ALS.

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P115 UNC13A GENOTYPE INFLUENCES SURVIVAL IN ITALIAN ALS PATIENTS

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Keywords: UNC13A, genetic

Objective: To evaluate the association of rs12608932, located within an intron of *UNC13A* gene on chromosome 19p13.3, with susceptibility and survival of ALS in a population-based cohort of Italian ALS patients.

Methods: A total of 500 ALS cases ascertained through Piemonte and Valle d'Aosta Register for ALS were included in the study. A total of 247 healthy subjects matched for age, gender and geographic origin were included in the study as controls. Samples were genotyped on Infinium

HumanHap550 beadchips or on Infinium HumanHap610-Quad beadchips.

Results: For our SNP of interest in *UNC13A* (rs12608932), 236 (47.2%) Italian patients carried the AA genotype, 203 (40.6%) the AC genotype and 61 (12.2%) the CC genotype. The corresponding frequencies among controls were 107 for AA (43.3%), 120 for AC (48.6%), and 20 for CC (8.1%). rs12608932 was not associated to ALS susceptibility ($p = 0.052$), but it was associated to survival under the recessive model (median survival for AA/AC 3.5 years, IQR 2.2–6.4; CC 2.5, IQR 1.6–4.2; $p = 0.017$) but not under the additive or dominant models. In Cox multivariable analysis rs12608932 genotype remained an independent prognostic factor (hazard ratio 1.40, 95% confidence interval, 1.05–1.87).

Conclusions: In our large population-based series including patients of Italian ancestry rs12608932 was not associated to ALS susceptibility but was strongly related to ALS survival with the minor allele carrier status displaying a ˜1-year reduction of survival under the recessive model. The identification of *UNC13A* as a determinant of progression rate of sporadic ALS opens a new scenario toward the discovery of therapies targeted at modifying the expression of *UNC13A*.

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P116 NOVEL MUTATIONS IN SOD1, TARDBP AND FUS IN CHINESE HAN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: gene mutation, China

Objective: Mutations in *SOD1*, *TADRBP* and *FUS* genes have been identified in both familial and sporadic amyotrophic lateral sclerosis (ALS) in several ethnic populations. However, so far, no large-scale screening of mutations in these genes has been reported. In fact, no studies have examined *FUS* mutations in ALS patients from mainland China. We systematically examined the spectrum of *SOD1, TARDBP* and *FUS* mutations and their frequencies in a Chinese ALS population.

Methods: 201 ALS patients (199 sporadic and 2 familiar) and 200 healthy controls from two centers in mainland China were analyzed by direct sequencing. Clinical manifestations of patients carrying different mutations were compared in our study.

Results: A total of 6 pathogenic missense mutations, 1 novel 3-bp insertion and 5 nonsense mutations were identified in our study: 3 in *SOD1* (G16S,V31A, V47A), 3 in exon 6 of *TARDBP* (A366A, A321A, G298S), and 6 in *FUS* (G49G, Y97Y, G246S, G228G, C.666_667insGCC, G497V). Among these, V31A, V47A, G246S, G228G, G497V are novel mutations. G49G and Y97Y were the only mutations also found in our healthy controls. The distribution of these mutations is consistent with (ACR1) the hypothesis of mutation hotspots in these genes. (ACR2) All 6 missense mutations are located in highly conserved sequences. Genotype-phenotype correlation analysis showed that site of disease onset (ACR3) and lifespan are two clinical symptoms that vary among ALS patients carrying missense mutations.

Conclusions: This study identified several new mutations associated with sporadic ALS in a Han Chinese (ACR4) population. This not only broadens the spectrum of disease mutations, but also underscores the importance of *FUS* and *TARDBP* (ACR5) as pathogenic genes alongside *SOD1*.

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P117 CHINESE SOD1 MUTATION SPECTRUM: GENOTYPE-PHENOTYPE CORRELATIONS IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Chinese, SOD1, spectrum

Background: Familial amyotrophic lateral sclerosis (FALS) is reported approximately 5%-10% of ALS cases in the world. Mutations in copper/zinc superoxide dismutase 1 (SOD1) are found in approximately 20% of FALS. As a huge country with big population, however, China has no data of SOD1 mutations in FALS so far.

Objective: The aim of this study is to identify the pattern of population distribution and correlations of SOD1 mutations and clinical phenotype in Chinese patients with FALS.

Methods: We collected the clinical data of 43 FALS families from 2008 to 2011, screened SOD1 gene mutations in the probands using PCR and direct sequencing and analyzed the correlations of genotype-phenotype.

Results: All 43 families were autosomal dominant inheritance who came from 17 provinces of China. The male female ratio of probands was 1: 0.6 and the average onset age $48.1 + 11.8$ years. Upper limb onset accounted for 53.5%, lower limb onset 41.9% and bulbar onset 4.6%. Seven probands (6 male and 1 female) died which caused by respiratory failure or pulmonary infection and the duration were 15–180 months ($73.3 + 64.2$ months). Nine SOD1 mutation types were detected in 10 probands including 8 missense mutations and 1 deletion mutation. Seven mutations were reported before, but six among them (C6S, G16A, E21G, G41D, G93R and L84F) detected first in Chinese patients. Two mutations (G16C and K128 fs X131) were novel including the first deletion mutation in Chinese FALS.

Conclusions: We reported the correlation of genotype-phenotype of SOD1 in a larger group of Chinese FALS patients. The detection rate of SOD1 mutations in this cohort was 23.3%. SOD1 mutations increased from 11 to 19 in Chinese FALS. For SOD1 patients, lower motor neuron (LMN) signs usually predominated. Two novel mutations were found including one deletion mutation. Other six mutations were first reported in Chinese FALS. Most clinical phenotypes of one mutation varied greatly in different families even the same family.

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P118 UBIQUILIN 2 MUTATIONS IN ITALIAN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

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Keywords: ubiquilin, UBQLN2, genetics

Background: Mutations in the *ubiquilin-2* (*UBQLN2*) gene have been shown to cause a familial form of X-linked amyotrophic lateral sclerosis (ALS), 90% penetrant in females (1).

Objectives: The aim of our study was to analyze the *UBQLN2* gene in a large cohort of familial and sporadic ALS patients, with or without frontotemporal dementia (FTD), and in patients with FTD.

Methods: We analyzed the *UBQLN2* gene in 819 sporadic ALS cases (SALS), 226 familial ALS cases (FALS), 53 ALS-FTD patients, and 63 patients with a clinical record of FTD. Molecular analysis of the entire coding sequence was carried out in all FALS and ALS-FTD patients, while SALS and FTD patients were analyzed specifically for the genomic region coding for the PXX repeat tract. Healthy controls were 461 anonymous blood donors and were screened for the PXX repeat region only.

Results: We found five different variants in the *UBQLN2* gene in five unrelated ALS patients. Three of them, including two novel ones, involved a proline residue in the PXX repeat region and were found in three FALS cases. The other two were novel variants, identified in one FALS and one SALS patient. None of these variants were present in controls, while one control carried a new heterozygous variant.

Discussion and conclusions: Our data support the role of *UBQLN2* gene in the pathogenesis of FALS, being conversely a rare genetic cause in SALS even when complicated by FTD.

Reference

1. Deng HX, Chen W, Hong ST *et al.* Nature 2011;477: 211–215.

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P119 UBIQUILIN2 MUTATIONS IN PARKINSON'S DISEASE EXTEND THE SPECTRUM OF UBIQUILINOPATHIES

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Keywords: Parkinson's disease, ubiquilin 2, mutations

Background: Ubiquilin2 belongs to the ubiquitin-like protein family which delivers uiquitinated proteins to the proteasome system (UPS) and autophagosome for protein and organelle degradation. Mutant ubiquilin2 recruits protein aggregates and become a pathological hallmark in several neurodegenerative diseases, such as ALS and ALS-dementia. To identify whether the ubiquilin2-positive inclusions is a common phenomenon in neurodegenerative diseases, Parkinson's disease (PD), the second most common in the world was chosen to screen for ubiquilin2 mutations. Two mutations were identified in PD patients. The effect of these mutations on the UPS and autophagy pathways was investigated via flow cytometry using Ub^{G76V}-GFP and LC3-GFP reporting system.

Objectives: We identified new ubiquilin2 mutations in 400 PD patients and 900 controls. Ub^{G76V}-GFP and LC3-GFP reporters were used to test the impairment of protein degeneration pathways in the two novel PD-related ubiquilin2 mutations.

Methods: Genomic DNA was PCR-amplified and sequenced by a CEQ-8000 system (Beckman Coulter). Immunohistochemistry and immunofluorescent stain were performed by standard protocol. Expression vectors of ubiquilin2-WT and the two PD-related mutations (M1 and M2) were co-expressed into Neuro-2A and SH-SY5C cell lines with either Ub^{G76V}-GFP or LC3-GFP reporters. Transiently transfected cells were analyzed by a BD LSRFortessa flow cytometer and BD FACSDiva softwarse.

Results: We sequenced 110 familial and 290 sporadic PD patients. M1 was found in familial cases and located between 2nd and 3rd STI1 motifs; and another mutation was found in sporadic cases and located in the 3rd STI1 motif (M2). They were absent in controls. Ubiquilin2 was found in Lewy bodies and Lewy neuritis in the substantia nigra in M1. The cleavage of GFP in UPS reporter with co-expression of the PD-related mutations in Neuro-2A cells showed no difference. An autophagosome marker (LC3-GFP) was used to test the impairment of autophagy pathway, which showed a tendency of impairment in M2. After we used SH-SY5C cells which have some dopaminergic charactersistics, the intensity of LC3-GFP fluorescence showed an 18% increase. Both mutations showed cell-type-related autophagosome impairment, $p < 0.0004$.

Discussion and conclusion: The novel PD-related mutations locate in different functional domains other than the reported PXX region mutations. The new mutations did not cause any disruption in proteasome system in vitro, while they exhibited cell specific autophagy impairment. The patient with M1 showed ubiquilin2-positive inclusions in dopaminergic cells. Our results demonstrate that ubiquilinopathies are etiologic and/or pathologic

factors underlying a wide spectrum of neurodegenerative diseases.

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P120 DE NOVO DELETION ON CHROMOSOME 4 (4Q22.1) IN THE GRID2 GENE CAUSES A NEW VARIANT OF SPASTIC PARAPLEGIA

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Keywords: GRID2, spastic paraplegia, mouse model

Background: Spastic paraplegia syndrome (SPG) subdivides into a sporadic and a hereditary form, the hereditary spastic paraplegia (HSP). The variable neurological syndrome comprises a spastic paraparesis with additional symptoms of ataxia, mental retardation and peripheral neuropathy in the complicated form. Clinical heterogeneity is partly explained by the 48 gene loci identified so far. We report a *de novo* deletion in the gene of the ionotropic glutamate receptor GRID2 that causes the clinical picture of a complicated SPG with cerebellar ataxia and mental retardation. GRID2 is expressed predominantly in the Purkinje cells in the cerebellum where it is involved in the postnatal linking with climbing fibers and the formation of synaptic plasticity. The only existing models for a gene defect in GRID2 are exclusively murine models (hotfoot, Lurcher), which present as cerebellar ataxia with a jerky movement disorder and retarded learning.

Objectives: To present a new variant of complicated spastic paraplegia where a mouse model already exists

Clinical Presentation: We saw a 21-year old man who upon examination presented with a spastic paraparesis that had been progressing over 10 years and a gait disturbance evident in a severely reduced walking distance. Dysarthria had been manifest for 3 years and dysphagia for 1 year. The neurological examination showed a high-grade spastic paraparesis with increased reflexes mainly in the legs, positive pyramidal signs, distal myatrophy of the upper extremities, and a cerebellar syndrome with intention tremor, dysarthria and dysphagia. Sensitivity was inconspicuous. Electrophysiological diagnostics showed signs of a chronic neurogenic reconstruction in all the muscles examined. The only finding in the MRI of the entire neuro axis was moderate cerebellar atrophy. Neuropsychological diagnostics revealed mild cognitive impairment.

Results: The chromosome analysis of the entire genomic array CGH substantiated an interstitial deletion of approx. 275 kb on chromosome 4q22.1 in the gene of the ionotropic glutamate receptors GRID2. Following qPCR, a corresponding deletion in the parents could be excluded.

Discussion and conclusions: The clinically heterogeneous HSP and its 48 identified gene loci are complemented by the *de novo* deletion in the GRID2 gene described here. A classic proof of heredity by inheritance over generations is superfluous as there is an analogy of symptoms in the mouse models known since the 1960s. Genetic proof of HSP in patients without former genetic confirmation of the syndrome can be facilitated through a targeted search in the GRID2 gene. Furthermore, the GRID2 mouse models may be applied in the research into therapy of degenerative diseases of the motor system.

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P121 EXOME SEQUENCING IDENTIFIES MUTATIONS UNDERLYING MOTOR NEURON SYNDROMES

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Keywords: exome sequencing, familial amyloidosis, ataxia

Exome sequencing has emerged as a powerful technique to identify the causes underlying monogenic disorders. It is also being increasingly utilized as a diagnostic tool, particularly in cases of genetic and clinical heterogeneity. Here we describe our application of exome sequencing to two American families with different motor neuron syndromes of uncertain classification. The first is an autosomal dominant bulbar onset neuropathy family. Exome sequencing revealed a mutation in *GSN* encoding Gelsolin (c.654g>t). This is the first reported American family with this mutation. The family was subsequently diagnosed with Familial Amyloidosis of the Finnish type. The second family is a consanguineous family with a novel motor neuron syndrome previously diagnosed as juvenile ALS via clinical examination. Exome sequencing now reveals a novel mutation in *SYNE1*(c.19821delG)-a gene in which mutations are known to cause autosomal recessive cerebellar ataxia type 1. Clinical re-examination of the family confirms that this syndrome is indeed ataxia. These findings highlight the utility of exome sequencing for rapid and comprehensive assessment in diseases that are rare, and for instances in which clinical manifestations of disease do not immediately provide clues as to the underlying genetic lesion.

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P122 A CASE OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS DUE TO MULTI-SYSTEM PROTEINOPATHY 1 AND HUNTINGTON DISEASE

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Keywords: VCP, familial ALS, Huntington

Objective: To clinically, electrophysiologically and pathologically describe a case of rapidly progressive neurodegenerative disease in an individual with a valosin containing protein (VCP) mutation and a Huntington (HTT) CAG repeat expansion.

Background: Mutations in the VCP gene have recently been identified as a cause of familial ALS broadening the phenotypic spectrum from inclusion body myopathy with

Paget disease and frontotemporal dementia (IBMPFD). The acronym IBMPFD is therefore insufficient to describe all disorders due to mutations in VCP and instead we favor the descriptor Multi-System Proteinopathy (MSP), using MSP1 for a disease associated with VCP mutations. VCP mutations result in defects in the protein degradation pathway. Huntington Disease (HD) is a monogenic disease caused by a repeat expansion in the HTT gene that in turn leads to abnormal protein aggregation.

Methods: A patient with Paget's disease and rapidly progressive neurological problems, whose father had Paget's disease and ALS and his mother had HD was examined clinically, electrophysiologically, genetically and followed until his death after which a detailed neuropathological examination was performed.

Results: The male patient presented at age 47, four years prior he had onset of cognitive and coordination problems which quickly progressed and choreatic movements appeared. He was known to carry a HTT repeat expansion of 44. The choreatic movements paradoxically diminished as weakness and amyotrophy developed. Clinically a diagnosis of ALS was made and an EMG demonstrated no myopathic changes, but widespread ongoing denervation and chronic reinnervation changes. A mutation in the VCP gene (R155H) was identified. The patient died 8 months later, only 4.5 years after his first behavioral symptom and 18 months after the first appearance of weakness. The specific neuropathological findings including RNA-binding proteins, TDP 43 and poly -Q inclusions are described.

Discussion: This is a unique case of a neurodegenerative disorder due to a VCP mutation combined with an HTT repeat expansion. The resulting phenotype was one of multi system neurodegeneration. The rapid disease course may be due to the pathological processes working synergistically, i.e., the VCP defect reducing the ability to degrade abnormal protein accumulations from the HD.

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P123 ASSOCIATION STUDIES OF MMP-9 IN PARKINSON'S DISEASE AND SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MMP-9 polymorphism, Parkinson's disease

Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) share several clinical and neuropathologic features, and studies suggest that several gene mutations and polymorphisms are involved in both conditions. Matrix metalloproteinase-9 (MMP-9) is implicated in the pathogenesis of PD and ALS, and the C(-1562)T polymorphism in the *MMP-9* gene leads to higher promoter activity. We therefore investigated whether this polymorphism predisposes to both PD and sporadic ALS (sALS). Samples from 351 subjects with PD and 351 healthy controls from two major cities in China were compared, while samples from 226 subjects with sALS were compared to the same number of controls from three centers in China. A possible association between the C(-1562)T polymorphism in the *MMP-9* gene and PD or sALS was assessed by restriction fragment length polymorphism (RFLP) analysis. Our results show a significant association between the C(-1562)T polymorphism in the *MMP-9* gene and risk of PD (odds ratio = 2.268, 95% CI 1.506–3.416, $p < 0.000$) as well as risk of sALS (odds ratio = 2.163, 95% CI 1.233–3.796, $p = 0.006$), supporting a role for *MMP-9* polymorphism in the risk for PD and sALS.

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P124 FIVE NOVEL MUTATIONS IN ALSIN GENE IN JUVENILE ALS AND JUVENILE PLS INDIVIDUALS

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Keywords: Alsin, mutations, JALS

Background: Majority of ALS cases are sporadic (SALS), but about 5–10% of the cases are familial (FALS). Eleven FALS loci at autosomes (ALS1 to ALS11) and one at X-chromsome (ALS-X) have been identified. SOD1 mutations account for 15–20% of all the FALS cases. Mutations in other ALS-related genes, such as senataxin, VAPB and Alsin are relatively rare. Only fifteen mutations in *Alsin* have been identified. Clinically, *alsin*-related diseases share many similarities. The mutations can be identified in ALS2, JALS, PLS and HSP individuals.

Objective: To identify new mutations in the *Alsin* gene in chronic juvenile amytrophic lateral sclerosis (ALS2) and related disorders.

Methods: Genomic DNA was used to conduct genetic analysis of the *Alsin* gene in nine patients from five unrelated families according to standard procedure. Pulse-chase study was performed for three of the six mutations to test the stability of mutant proteins.

Results: Five of these six mutations are novel, which include one each of amino acid substitution (p. R1610W) and a single amino acid deletion (p.120delA), and three mutations that cause a frame-shift resulting in truncated alsin (p. G477AfsX19, p. D1517RfsX26 and p. T512HfsX46). All the five novel mutations have been found in patients with infantile to juvenile-onset primary lateral sclerosis (IJPLS). A previously reported frameshift mutation (p. A47fsX4) has been identified in a patient with ALS. Pulse-chase studies of three representative mutants including p.120delA, p. R1610W and p. T185fsX5 demonstrated that decreased protein stability is a common feature, but the least stability is observed in truncation mutant.

Discussion and conclusions: Our data indicates that the position and type of mutations are important factors for the clinical spectrums of the patients with the *Alsin* mutations. It

appears that disruption of the first four RCC1 domains is essential for involvement of lower motor neuron resulting ALS other than PLS phenotype. Based on our data and literature review, we proposed a term "infantile to juvenile-onset primary lateral sclerosis (IJPLS)" to describe the primary lateral sclerosis that has infantile to juvenile -onset such as that caused mutations in the *Alsin*.

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P125 LATE ONSET TRIPLE-A SYNDROME WITHOUT ADRENAL INSUFFICIENCY MIMICKING JUVENILE ALS: A FURTHER CASE AND LITERATURE REVIEW

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Keywords: young onset ALS, triple A sydrome, differential diagnosis

Background: Triple A syndrome (Allgrove syndrome OMIM#231550) is an autosomal recessive condition characterized by alacrima, achalasia, ACTH-resistant adrenal insufficiency, autonomic dysfunction and neurodegeneration. The syndrome usually begins in childhood but may appear in the third decade with an incomplete phenotype.

Objective: We present here a 36-year-old male referred to our clinic due to progressive weakness in the hands over five years. He referred the guide sign of a history of dysphagia to solids since 10 years of age.

Material and methods: Clinical characterization, EMG and adrenal blood screening, including plasma concentration of ACTH, cortisol, DHEA-S and aldosterone were performed. Genetic testing after receiving informed consent included collection of blood samples for screening for causes of juvenile ALS: SOD1, FUS, TDP43, AAAS, dynactin, and CAG repeats.

Results: Neurological examination showed symmetrical amyotrophy, predominantly in the thenar and hypothenar eminence, and in the forearm distal muscles to some extent. The reflexes were brisk, with Hoffman, Trömner and bilateral Babinski signs. Nasal voice, bulbar dysarthria and hand tremors were also observed. Tongue protrusion was weak. Electromyography showed signs of chronic denervation in the muscle of the four extremities, which was more severe in the distal muscles. TMS showed a central conduction time of 35.0 msec for the right tibialis anterior and 16.4 msec for the right abductor digiti minimi. The laboratory examination revealed normal plasma levels for ACTH, normal serum levels for cortisol, Dehydroepiandrosterone, 17-Hydroxyprogesterone, aldosterone and normal serum electrolyte levels. The patient was a compound heterozygote with two previously described mutations: c.211delC, and c.43C>A.

Discussion: Our findings further expand the already wide spectrum of the phenotype associated with Allgrove syndrome, and should be included in the differential diagnosis of juvenile onset patients presenting with upper and lower motor neuron signs. The case described here is the first Spanish case to be reported with clinical and laboratory data.

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P126 ATXN2 INTERMEDIATE-LENGTH CAG REPEAT EXPANSIONS INCREASE THE RISK FOR ALS IN MAINLAND CHINA

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Keywords: ATXN2, polyglutamine, China

Background: 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.

Objectives: In this study, we proposed to analyze the *ATXN2* CAG repeat length in Chinese patients with SALS and to find the relationship between the genotype and phenotype.

Methods: 432 patients with SALS and 473 matched controls from Mainland of China (except Tibet) were studied. All the patients were diagnosed with ALS, fulfilling the revised El Escorial criteria for definite, probable or lab-supported probable ALS. We collected the clinical data and analyzed the fluorescence PCR products to assess the *ATXN2* CAG repeat length in all the samples.

Results: In SALS, the maximal *ATXN2* CAG repeat size was 34, while it was only 30 in controls. We observed that intermediate CAG repeat expansions in *ATXN2* (CAG repeat length $\geq$ 29) were associated with SALS ($p = 0.037$; OR: 2.53; 95% CI: 1.036, 6.182). There was no significant difference in the clinical characteristics between the group with and without intermediate CAG repeat expansions in *ATXN2*, such as gender, age of onset, site of onset, brainstem involved, levels of diagnostic certainty, fasciculation and ALS-FRS.

Discussion: In line with the previous studies, intermediate CAG repeat expansions in *ATXN2* are associated with SALS, whereas the specific cutoff discriminating ALS from control varies in different population. Unlike the strong inverse correlation between age of onset and repeat length in SCA2, the repeat length does not influence the clinical characteristics in ALS, which suggests that the mutant types of the *ATXN2* gene determine the pathogenic mechanisms in different disorders.

Conclusions: Our data indicated that for SALS patients from Mainland of China, intermediate CAG repeat expansions in *ATXN2* increase the risk of the disease and have no effect on the phenotype of ALS.

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P127 ZNF512B GENE SERVES AS A PROGNOSTIC FACTOR IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: single-nucleotide polymorphism , ZNF512B, prognostic factor

Background and objective: Recently, the study team led by Iida *et al.* conducted a large-scale genetic association study in 1305 Japanese patients with amyotrophic lateral sclerosis (ALS) and 4244 controls, discovering a new single-nucleotide polymorphism (SNP) associated with susceptibility to ALS in the ZNF512B gene. Although ALS susceptibility genes had already been discovered in Europe and the United States, the present analysis involving Japanese subjects resulted in the first discovery of the ALS susceptibility genes in East Asians. Because the study on the ZNF512B gene and ALS conducted by Iida *et al.* was based mainly on *in vitro* analysis results, what influence the new SNP exerts on actual ALS patients remained unknown. Thus, we analyzed the ZNF512B gene in patients diagnosed as having ALS and reviewed and evaluated both results and clinical manifestations.

Methods: We analyzed the ZNF512B gene in 164 patients diagnosed as having ALS in the past 15 years at our hospital and retrospectively reviewed and evaluated both results and clinical manifestations.

Results: The risk allele of the new SNP was detected in 122 (74.4%) of these 164 patients with ALS. The mean age at onset of ALS was 63.6 years in patients without the risk allele and 63.7 years in those with the risk allele, showing no significant difference. However, regarding the prognosis after onset, Kaplan-Meier survival curves for patients with versus without the risk allele revealed a significantly shorter survival in those with the risk allele (log-rank test, $P < 0.05$).

Discussion and conclusion: The protein encoded by the ZNF512B gene is a transcription factor promoting expression(s) of a downstream gene(s) in the signal transduction pathway of TGF-β, which is essential for the protection and survival of neurons, and ZNF512B gene expression is also reportedly increased in motor neurons of ALS patients. Taking these reports together with our data, it is clinically reasonable to suggest that the ZNF512B gene may also serve as a new prognostic factor in ALS.

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P128 PATHWAY AND GENE SET ANALYSIS OF GENOME WIDE ASSOCIATION STUDY ON SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: single nucleotide polymorphism, gene set and pathway analysis, susceptibility

Background: Genome-wide association studies (GWAS) testing several hundred thousand SNPs have been performed in ALS and other complex diseases. Typically, the number of SNPs in which the evidence for association exceeds the genome-wide significance threshold is very small, and SNPs that do not exceed this threshold are generally neglected. Pathway and gene set analysis of genome wide association study provides a means of integrating the results of a GWAS on ALS and the genes in a known molecular pathway to test whether the pathway is associated with the disorder.

Objective: We hypothesize that certain combinations of genes flagged by these SNPs can be identified if they belong to a common biological pathway.

Methods: We conduct a pathway-oriented analysis of six GWAS in ALS that takes into account all SNPs with nominal evidence of association ($P < 0.05$). Gene set P-values were superimposed on a human protein interaction network and searches were conducted to identify sub-networks containing a higher proportion of genes associated with ALS than expected by chance. These sub-networks, and others generated at random as a control, were categorized for membership of biological pathways. GWAS from four other diseases were analyzed to assess the specificity of the pathways identified. Several well know software and database be adopted.

Result: In the ALS datasets, we identified sub-networks of genes from several pathways including axonal transport which previously identified. We report here for the potential involvement of inflammation, mitochondrial and autophagy pathways in ALS susceptibility.

Conclusion: Multiple SNP and pathway are involved in ALS susceptibility. The result should be carefully interpreted.

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P129 A FUNCTIONAL VARIANT IN THE PON1 GENE IS ASSOCIATED WITH SHORTER SURVIVAL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: paraoxonase, genetics, association study

Background: The paraoxonases (PON) are a family of enzymes involved in preventing lipid membrane peroxidation, and in detoxification of exogenous compounds. Several

studies have suggested an association between single nucleotide polymorphisms (SNP) in the PON genes and increased risk of developing amyotrophic lateral sclerosis (ALS).

Objectives: Our study aims to assess whether the functional SNP rs661 in PON1, which alters the substrate specificity of the enzyme, has an effect on disease phenotype.

Methods: 341 Italian patients with sporadic ALS were genotyped using the Human 660W-Quad BeadChip (Illumina), and rs661 genotypes obtained with the GenomeStudio software. Phenotypic traits analyzed included: age at onset, site of onset, distribution and severity of muscular involvement, global functional impairment, disease duration and the time from onset to the start of non-invasive ventilation (NIV).

Results: In comparison with patients homozygous for the major allele (A) of rs661, individuals with at least one copy of the G allele presented more frequently with a bulbar (28.8% vs 19.3; $p = 0.01$) or respiratory onset (10.7 vs 1.6%; $p < 0.01$). Also, GG patients often showed a symmetrical (48.1% vs 19.8%; $p < 0.01$) and proximal (50.0% vs 27.6%; $p = 0.04$) distribution of the motor deficit. Lastly, individuals homozygous for the G allele had a shorter time-to-NIV (14.3 vs 31.2 months; $p = 0.01$), and reduced survival (26.8 vs 41.4 months; $p < 0.01$).

Discussion and conclusions: The G allele of rs661 may increase the susceptibility of motor neurons innervating bulbar and axial muscles to neurodegeneration in ALS, ultimately resulting in a faster respiratory decline and shorter survival.

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P130 POLYGENIC VARIATION IS NOT ASSOCIATED WITH AGE AT ONSET IN GENOME-WIDE ASSOCIATION STUDIES OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: age at onset, single nucleotide polymorphism, genome-wide association

Background: Previous studies have explored the effects of monogenetic variation on phenotypic characteristics of ALS, with contradictory results. Genome-wide association studies (GWAS) allow estimation of polygenic effects of single nucleotide polymorphisms (SNPs) on disease. A recent GWAS used a method of 'scoring' cases based on numerous 'score alleles', demonstrating the contribution of polygenic variation towards developing schizophrenia (1).

Objectives: We adapted this method to look for associations between SNPs and age at onset (AAO), in GWAS data from several countries, to examine whether polygenic variation contributes to AAO of sporadic ALS (SALS).

Methods: We analysed SNP and AAO data on Caucasian male and female SALS cases from four countries separately: the UK, Ireland, the Netherlands and USA, and a 'Combined' sample of all cases ($n = 2641$). GWAS methods were as previously described (2–6). For each analysis we divided cases into discovery and independent target samples. We carried out linear regressions in discovery samples of normalised (squared) AAO values against reference alleles at 74,011 genotyped independent SNPs, and 'scored' target sample cases using significantly associated reference alleles, at decreasingly stringent overlapping P value thresholds (P_T). We used linear regressions in corresponding target samples to estimate the degree of variation in AAO attributable to scores (representing polygenic effects) at each P_T. All analyses were adjusted for gender, and in the 'Combined' sample, for principal components for ancestry.

Results: In separate and 'Combined' target samples, we found no statistically significant associations between scores and AAO at any P_T. At certain P_T ($P_T < 0.01$, $P_T < 0.05$) we occasionally obtained marginally significant results ($0.01 < P < 0.05$). However, on reproducing these analyses several times for multiple discovery/target sample divisions at each P_T, all results became non-significant ($P > 0.05$).

Discussion and conclusion: Our results suggest that polygenic variation may not affect AAO of SALS. This may be because AAO is determined by environmental factors. Alternatively, as GWAS involve analysing combined effects of multiple, small genetic variations, our power to detect individual variations that subtly influence AAO may be limited at our sample size. Finally, our P_T may not have allowed detection of the full variety of potential polygenic models. Future work should extend our approach to larger GWAS samples, using a wider variety of P_T, in sporadic and familial ALS.

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P131 CONFOCAL MICROSCOPY ANALYSIS OF ALTERED TDP-43 EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTES FROM ALS PATIENTS

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Keywords: peripheral blood mononuclear cells, TDP-43, confocal microscopy

Background: Significant advances in gene expression studies in whole blood, peripheral blood mononuclear cells (PBMCs) and peripheral blood lymphocytes (PBLs) have been made recently. These studies identified specific alterations that may permit longitudinal assessment of potential biomarkers with respect to treatment effects in prospective clinical trials. Analyses of whole-genome transcriptional expression and TAR DNA-binding protein 43 (TDP-43) expression in freshly isolated or sub-cultured PBMCs or PBLs, have indicated that the peripheral blood compartment, in particular, the PBL and PBMC components, may partially replicate pathophysiological changes occurring in the central nervous system of ALS patients.

Objective: Our objective was to investigate changes in non-nuclear and nuclear expression of TDP-43 in PBLs from ALS patients treated or not treated with riluzole (Rilutek) compared to healthy controls (HCs).

Methods: PBMCs were collected from ALS patients and HCs at an academic medical center-based ALS clinic. Anti-TDP-43 antibody was used for immunocytochemistry. A Zeiss LSM 710 confocal microscope was used to determine TDP-43 non-nuclear and nuclear staining in PBLs from ALS patients and age and gender-matched HCs.

Results: Non-nuclear expression of TDP-43 is increased in PBLs from ALS patients not treated with riluzole (n = 14) compared to HCs (n = 15) and to a lesser extent in ALS patients treated with riluzole (n = 12). Changes in non-nuclear expression of TDP-43 in PBLs from ALS patients did not correlate with disease duration (onset to sampling), ALSFRS-R or rate of progression.

Discussion and conclusion: Confocal microscopy is a useful tool to assess TDP-43 expression in PBLs from ALS patients as a potential biomarker for diagnosis, rate of progression and response to therapy. However, responses produced by lymphocytes in ALS patients may be highly heterogeneous. Investigation of ALS-specific mechanisms involved and the generation of longitudinal data are necessary to establish reliable correlations.

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P132 ALTERNATIVE GENE SPLICING IDENTIFIED IN TDP-43 TRANSGENIC MICE IS ALSO PRESENT IN ALS PATIENTS

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Keywords: TDP-43, mRNA processing, mouse model

Objective: TDP-43 is an RNA binding protein known to regulate pre-mRNA splicing. The aim of this study was to determine whether mutation of TDP-43 results in alternative gene splicing in a transgenic mouse model of ALS, and whether ALS patients show similar changes.

Methods: Total RNA was extracted from the brain and spinal cord of mice that overexpress human TDP-43 containing an ALS causing mutation (A315T). Mice were studied before (50 days old) and after (100 days old) the onset of symptoms. Extracted RNA was hybridized to Affymetrix Exon arrays to facilitate the detection of alternative splicing. The presence of alternatively spliced transcripts was confirmed by RT-PCR, in both TDP-43 mice and ALS patient brain tissue.

Results: Pre-mRNA splicing of approximately 800 transcripts, previously identified as RNA binding partners of TDP-43, were affected by the presence of the A315T mutation. The splicing of more than 100 genes was altered prior to symptom onset. This increased to over 700 genes affected after the onset of symptoms. Biological functions associated with these genes included cell death and nervous system development. Using primers designed to amplify specific gene isoforms, alternative splicing detected by the microarray analysis was confirmed in an independent set of TDP-43 transgenic mouse samples. Alternative splicing of a subset of genes has also been confirmed in ALS and FTD patients, with and without TDP-43 mutations.

Discussion and conclusion: The exon array analysis revealed that expression of mutant TDP-43 affects global mRNA processing in the mouse brain and spinal cord and that alternative splicing of several genes precedes the onset of symptoms in this mouse model of ALS. The results suggest aberrant pre-mRNA splicing is one of the disease mechanisms that precede symptoms of ALS in TDP-43 transgenic mice. We are continuing to validate these findings in ALS and FTD patient tissue.

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P133 SPORADIC JUVENILE AMYOTROPHIC LATERAL SCLEROSIS CAUSED BY MUTANT FUS/TLS: POSSIBLE ASSOCIATION OF MENTAL RETARDATION WITH THIS MUTATION

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Keywords: juvenile ALSFUS/TLS, mental retardation

Background: Mutations in the fused in sarcoma/translocated in liposarcoma (FUS/TLS) gene have been shown to be responsible for ~3% of familial ALS (FALS) and < 1% of sporadic ALS

(SALS). FUS/TLS-positive neuronal intranuclear inclusions and glial cytoplasmic inclusions have been found not only in ALS but also in neuronal intermediate filament inclusion disease (NIFID), atypical frontotemporal lobar degeneration (FTLD) with ubiquitinated inclusions (aFTLD-U), and basophilic inclusion body disease (BIBD). FUS/TLS is likely to play a pivotal function in motor neurons as well as in more extensive regions of central nervous systems, but the exact mechanism remains unclear.

Objectives: We encountered 2 patients with sporadic juvenile ALS with mutations of the FUS/TLS gene. Gene analyses revealed mutations of p. G492EfsX527 (c. 1475delG), which is a novel deletion/frameshift mutation, in the first patient and p. R514S mutation (c. 1542G>T) in the second patient. The aim of this study is to clarify the clinical phenotype in the both patients, and to determine the mechanism by which the deletion/frameshift mutation caused neuronal dysfunction.

Methods: We assessed subcellular localization of these FUS/TLS mutants in transfected motor neuron-like cells, compared with wild-type FUS/TLS.

Results: The symptoms of both patients started as weakness of the unilateral upper limb and neck, and extended to bulbar and respiratory weakness in a relatively short period. Both patients had no behavioral or personality changes suggestive of frontotemporal dementia. Of note, the first patient had suffered from mental retardation before the onset of weakness. Molecular analysis revealed that the mutant FUS/TLS, especially the deletion/frame shift mutation, showed significant cytoplasmic localization in transfected motor neuron-like cells. More interestingly, the FUS/TLS mutants not only localized to the cytoplasm, but also formed nuclear aggregates.

Discussion and conclusions: Our findings suggest the association of mental retardation with the FUS/TLS mutation. Further investigation, including the effect of FUS/TLS on cognitive function, would aid better understanding of FUS/TLS proteinopathies.

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THEME 6 EPIDEMIOLOGY

P134 GLOBAL EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS (ALS): A SYSTEMATIC REVIEW OF THE LITERATURE

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Keywords: incidence, prevalence, systematic review

Background: ALS is relatively rare, yet the economic and social burden is enormous. Having accurate incidence and prevalence estimates would facilitate efficient allocation of healthcare resources. To this end, it is important to understand the strengths and limitations of the epidemiologic literature and to account for the geographic and demographic diversity of the ALS population.

Objective: To provide a comprehensive and critical review of the epidemiologic literature on ALS.

Methods: MEDLINE and EMBASE (1995–2011) were searched to identify English-language, population-based, observational studies on the incidence and/or prevalence of ALS. Information extracted included: location and time period, study design and data sources, diagnostic criteria and case ascertainment methods, and incidence and/or prevalence rates. Medians and inter-quartile ranges (IQRs) were calculated for studies by region, and case estimates were calculated using 2010 population estimates.

Results: 1,572 publications were identified, 155 articles were retrieved for review. Most of these were excluded because reported only mortality or risk factor data (n = 88). When the same population was evaluated in ≥ 1 studies (n = 12), the most recent article was retained. Five studies were excluded as not population based and 8 because they did not provide sufficient data. Guam-based studies (n = 4) were also excluded. Overall, 38 articles met the inclusion criteria (20 prospective, 18 retrospective). Most studies were conducted in Europe (n = 26). There were 4 primary approaches to data collection: records' review (n = 16), ALS registries (n = 11), surveys (n = 6) and databases (n = 5). Most records' reviews were retrospective (81%); most registry- (73%) and survey-based studies (83%) were prospective. Most studies (68%) used multiple sources for case ascertainment. The most frequently used source was hospital records/databases (17%), followed by neurology departments/clinics (16%), and death certificates (12%). In Europe, the median (IQR) incidence rate (/100,000 persons) was 2.00 (1.37–2.40), corresponding to an incident case estimate of 14,764 (10,113–17,717). The median (IQR) prevalence rate (/100,000) was 6.30 (4.49–7.93), corresponding to a prevalent case estimate of 46,507 (33,145–58,539). Incidence/prevalence rates were higher among prospective versus retrospective studies. Preliminary evaluation of age-specific data showed a trend toward an increase in incidence with age.

Conclusions: The body of literature on the epidemiology of ALS is not small, but there is much disparity among reports. This may be due to differences in study design, including case ascertainment methods. Other factors include inter-country variations in age distributions and changes in these distributions over time. It is important to understand and account for this when reviewing the literature, as it may not accurately reflect the global burden of ALS. There is a clear need for large-scale epidemiological studies of ALS that utilize standardized approaches to case ascertainment.

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P135 INCIDENCE OF NEUTROPENIA AMONG HOSPITALIZED ALS PATIENTS IN A U.S. HEALTH INSURANCE CLAIMS DATABASE

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Keywords: comorbidities, neutropenia

Background: Neuroinflammation and other neuroimmune abnormalities have been previously reported in ALS (1). Emerging evidence also suggests abnormalities are found in peripheral whole blood cells; however, it is unclear if these peripheral blood cell abnormalities are associated with clinical hematologic abnormalities, such as neutropenia. Administrative medical claims are useful for estimating incidence rates of rare outcomes like neutropenia. Previous studies have shown inpatient neutropenia medical claims are more accurate than outpatient neutropenia medical claims (positive predictive value: inpatient = 97%, outpatient = 3%–56%) (2,3). However, the sensitivity of neutropenia medical claims is low (1%–35%) (3). Therefore, medical claims are likely useful for estimating a relative risk of clinically significant neutropenia, but may underestimate the true incidence rate.

Objective: Estimate the risk of neutropenia events in hospitalized ALS patients compared to controls within the i3 InVision Data Mart Multiplan database.

Methods: Two cohorts of patients, ≥ 18 years of age with no medical claims related to HIV, malignancies, or chemotherapy were included in this analysis: ALS patients (n = 1382, patients with 1 inpatient or 2 outpatient medical claims containing ICD-9 code 335.20) and controls (n = 65,000 randomly selected patients with no medical claims for ALS (ICD-9 code 335.20) or other motor neuron diseases (ICD-9 codes 335.2, 335.21, 335.22, 335.23, 335.24, 335.29)). The narrow neutropenia definition was any inpatient or emergency room medical claim containing ICD-9 code 288.00 (unspecified neutropenia). The broad neutropenia definition also included any inpatient or emergency room medical claim containing ICD-9 codes 288.03, 288.09, 288.5, 288.50, 288.59, 288.8, 288.9. Poisson regression

was used to calculate incidence rates while Cox proportional hazards models were used to calculate hazard ratios (HR).

Results: The narrow neutropenia definition did not identify any events in ALS patients; whereas 9 events were identified in controls (cumulative incidence = 0.01%). The cumulative incidence of neutropenia using the broad definition was 0.66% in ALS patients and 0.04% in controls. The crude incidence rate of neutropenia in ALS patients and controls using the broad definition was 463.2/100,000 person-years (PYs) and 46.1/100,000 PYs, respectively. The HR of neutropenia in ALS patients was 10.2 (95% CI: 4.8, 21.5). Results from the multivariate models will be presented at conference.

Discussion and Conclusions: In a medical claims database, ALS patients were at increased risk for clinically significant neutropenia. The reported incidence rates likely underestimate the true incidence rates in these populations given the poor sensitivity of the neutropenia claims definition. Future studies should explore whether neutropenia is associated with specific peripheral blood cell abnormalities previously reported in ALS.

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P136 STATE-BASED SURVEILLANCE FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: surveillance, epidemiology, prevalence

Background: The Agency for Toxic Substances and Disease Registry (ATSDR) launched the National ALS Registry to identify ALS cases using two main data sources: existing national administrative data (Centers for Medicaid and Medicare Services (CMS), the Veterans Health Administration (VHA), and the Veterans Benefits Administration (VBA)) and self-registration through a secure web portal. This non-traditional approach to case ascertainment needs to be validated against more conventional surveillance techniques.

Objectives: Develop state based surveillance programs to evaluate the completeness of the National ALS Registry.

Methods: With an active case-finding methodology similar to that of state-based cancer registries, the state-based surveillance activities rely on reports from medical providers and medical records abstraction rather than administrative data. Persons diagnosed with or provided care for ALS from January 1, 2009 through December 31, 2011 were included. Data from these sources will be used to evaluate the completeness of the National ALS Registry, with special attention to demographic characteristics of persons with ALS.

Results: Florida, New Jersey, and Texas, participated in the project. Combined, these three areas are 71.7% White, 13.6% African-American, 4.1% Asian, and 28.9% Hispanic. Compared with the US population, Hispanics are over-represented and African-Americans are slightly over-represented. Of the 2417 neurologists identified who might treat patients in these areas, only 598 (24.7%) had practices that would diagnose or care for a person with ALS. Through April 30, 2012, 3997 case reports were received. Demographic and diagnostic characteristics of the reported cases will be discussed.

Discussion and conclusions: Because of the unique way in which cases of ALS are identified for inclusion in the National ALS Registry, it is important to assess its completeness. States were selected to over-represent some racial and ethnicity minorities because of concerns related to possible difference in access to medical care and support organization resources. Differences in access to specialized medical services in each site complicate obtaining an accurate count of neurologists who diagnose or treat persons with ALS. In each state there are a handful of major treatment centers seeing 50 or more persons with ALS and only about 25%of neurologists diagnose or care for persons with ALS. All major treatment centers within participating states reported cases; however, case reporting from smaller practices made up almost 50% of the cases. This project demonstrates the difficulties in designing a surveillance system based on physician reporting, particularly for a non-reportable disease.

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P137 PROJECTED NUMBER OF CASES OF AMYOTROPHIC LATERAL SCLEROSIS IN THE UNITED KINGDOM UNTIL 2025: EXTRAPOLATION FROM A POPULATION BASED REGISTER

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Keywords: population register, incidence, burden

Background: Understanding the numbers of people affected by ALS now and in the future will enable planning of resources for delivery of care and the design of clinical trials. The South East ALS (SEALS) register captures incident cases in the South East of England (1–2).

Objectives: We used our register to estimate the burden of ALS in the UK and to project the figures for 5,10 and 20 years' time.

Methods: Incidence and point prevalence were calculated using data from 1997–2009. Population projections for the U.K. were obtained for every year to 2025 (3). The rate of ALS was calculated for each age cohort (broken into 15 year bands) and sex. The expected number is the product of the projected UK population and the proportion of ALS cases in the SEALS register for each age and sex cohort. The sum over all ages and both sexes gives the age and sex adjusted projected rate. The number of expected deaths was calculated in a similar manner.

Results: There were 483 patients registered between 2002–2009 who fell within the catchment area (255 ma

of 26,071,600 person-years of follow-up, giving a crude incidence of 1.8 per 100,000 person years for this period (95% CI 1.69–2.02) and an adjusted incidence rate of 1.49 per 100,000 (95% CI 1.35–1.64 per 100,000). There were 206 patients (106 males) alive on 31 December 2009 giving an adjusted point prevalence of 6.49 cases per 100,000 (95% CI 6.29–6.69). Using the point prevalence estimates for the SEALS region, the estimated number of people living with ALS in 2012 in the UK is 4094 (95% CI 3967–4352). This figure will rise to 4753 in 2025 solely due to changes in population structure (95% CI 4599–4887). The number of new cases of ALS in the UK that will be diagnosed between 2012 and 2025 is estimated to be 15,773 (95 % CI 15492–15952). Based on these calculations, there are 1,126 new ALS cases and 922 deaths per year.

Discussion and conclusions: The number of ALS cases will rise as a result of the change in population structure over the next 14 years. Aging populations in developed countries are also likely to follow this trend. The projected increase is the equivalent of the workload of three to four tertiary referral centres and is likely to be a conservative estimate. Any treatment that improves survival will dramatically increase the prevalence rates of ALS further.

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P138 AMYOTROPHIC LATERAL SCLEROSIS IN EMILIA ROMAGNA, ITALY: A POPULATION BASED STUDY FROM 2009 TO 2011. THE EMILIA-ROMAGNA REGISTER FOR ALS (ERRALS)

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Keywords: population based study, incidence, phenotypes

Background: In previous retrospective epidemiological studies performed in Emilia Romagna provinces the reported incidence rates ranged from 0.98 to 2.16/100,000. In Italy the existing population based registries showed incidence rates ranging from 1.6 in Apulia region (Southern Italy) to 2.5 in Piedmont and Valle d'Aosta region (Northern Italy). We report the first results from a prospective population-based epidemiological study in Emilia Romagna Region to describe the incidence and the clinical features and phenotypes of ALS from 2009.

Methods: This study was performed in 9 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 according to the revised El Escorial diagnostic criteria.

Results: From 1 January 2009 to 31 December 2011 in Emilia Romagna 340 patients (181 M, 159 F) received a new diagnosis of ALS (M: F ratio 1.14) with a crude average annual incidence rate of 2.58 cases per 100 000 person-years. Mean age at diagnosis was 69.73 years (range: 27–96 y). Mean diagnostic delay was 12.41 months. The onset was spinal in the 62% of cases. ALS was definite in 37%, probable in 47% and possible in 16% of patients at the moment of data entry. Dementia was present in the 7.35% of cases, whereas Parkinsonism was present in 1% of cases. ALS phenotypes were represented as follows: classic ALS 38.5%, bulbar ALS 35.6%, flail leg/arm 20.4%, Upper Motor Neuron Predominant 5.6%.

Discussion and conclusions: We report incidence rates similar to those reported by recent European population based studies. Also clinical features are similar to those described in previous population-based studies. The main strength of this study is the high incidence rate, which reflects a great accuracy in case ascertainment, and the large geographical area and population. These results confirm that population based studies are a valid tool to better define and characterize ALS population.

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P139 FREQUENCY OF PSEUDOBULBAR AFFECT (PBA) IN AN INCIDENT ALS COHORT: RESULTS FROM A POPULATION BASED REGISTRY

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Keywords: Pseudobulbar affect, Center for Neuroogic-Study Lability Scale, population-based registry

Background: Pseudobulbar affect (PBA) is a disorder of altered expression of emotions characterized by sudden and involuntary outbursts of crying and/or laughing either unrelated or disproportionate to the patient emotional state. It can occur in patients with many neurological disorders. Clinical studies have reported that it affects up to 50% of patients with ALS as the disease progresses. Despite its prevalence and its impact on the patients' quality of life, PBA is often underrecognised and undertreated. No data are available from incident cohorts of population-based studies.

Objectives: To evaluate the frequency of PBA in an incident cohort of ALS patients enrolled in a population

Methods: Incident cases, diagnosed in the 2011 period as having ALS according to the El Escorial criteria, were enrolled from a prospective population-based registry established in Puglia, Southern Italy. Neurological status was assessed at entry by a standard neurological examination and by the revised ALS Functional Rating Scale (ALSFRS-r). The Center for Neurologic Study-Lability Scale (CNS-LS) was used to evaluate the presence and the severity of pathological laughing and crying. It is a 7-item self-administered questionnaire to investigate PBA validated in ALS. Total scores range from 7 to 35. A cut-off score ≥ 13 was used to identify pathological cases.

Results: We enrolled 43 sporadic incident ALS cases, median age of 61 years (range: 33–77). Median onset-diagnosis interval (ODI) was 11 months (range: 2–69), median ALSFRS-r at baseline 39/48 (range: 14–47); median ALSFRS-r bulbar score was 21/24 (range: 6–24). At entry 27/43 patients (63%) showed bulbar signs of upper motor neuron (UMN) impairment and 38/43 (88%) presented bulbar lower motor neuron (LMN) involvement. Median total CNS-LS score was 9/35 (range: 7–25), with a median laughing sub-score of 4/20 (range: 4–16) and a median crying sub-score of 5/15 (range: 3–12). Pathological CNS-LS score was found in 15/43 patients (35%). There was a negative correlation between total CNS-LS score and ODI ($p = 0.042$; r_s: -0.312). CNS-LS score did not correlate with ALSFRS-r total and bulbar scores. Chi square test revealed that pathological CNS-LS was associated with presence of signs of UMN involvement ($p = 0.002$).

Discussion: This is the first report evaluating the presence of PBA in an incident cohort of patients with ALS. Our study showed that PBA is present in more than one third of patients with ALS also at the beginning of the disease and is related to UMN involvement.

Conclusion: This study shows that PBA may be present in patients with ALS independently from the severity of motor impairment. Therefore the evaluation of PBA should be part of the clinical assessment at entry.

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P140 ASSESSING AMYOTROPHIC LATERAL SCLEROSIS/MOTOR NEURONE DISEASE PATIENTS' SURVIVAL RATE CONCERNING EPIDEMIOLOGICAL, CLINIC ASPECTS AND RILUZOLE USE

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Keywords: epidemiology, survival, riluzole

Objectives: To analyze survival of patients with ALS/MND disease in relation to the epidemiological aspects (age, gender and race); the clinical features; the follow up since the initial symptom of the disease, the electroneuromyography; the initial topography involving the clinical impairment; the use of riluzole until the first appointment at Department of Research in Neuromuscular Diseases at Federal University of São Paulo (UNIFESP).

Method: This study is based on records (epidemiologic, physiopathologic, clinic), including subsidiary test results from Department professionals, from 1999 to 2011.

Results: We assessed 1,146 ALS/MND patient records and included 578, in which there was all the data according to the inclusion criteria. We assessed 231/578 patients (217 men, 104 women) for survival rate with the death date, who had used or had not used riluzole. These patients were divided into two groups, according to the confidence interval (95% lower), that is shown in the Kaplan-Meier survival curve: first: 144 deceased patients < 9 months for survival time; second: 87 deceased patients ≥ 9 months for survival time.

Conclusion: For the ALS/MND patients' survival rate analysis, epidemiological sex results showed there was no statistically significant difference. However, younger patients live more and there is too much time between the first symptoms and the first consultation. The patients who presented higher survival rate a) were whose without diagnosis in the first consultation, because there was no clinical impairment or there were no electroneuromyographic findings with the right diagnosis for them (and they also showed initial symptoms lower limbs) and b) used riluzole. The post-diagnosed patients that live over nine months seem to be more favoured by the use of riluzole. Thus, we can say riluzole is neuroprotective for individuals with more viable neurons.

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P141 GENDER AS A RISK FACTOR IN A SPANIARD ALS POPULATION

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Keywords: gender, estrogen

Background: Gender, with a higher incidence in men, is a well-known risk factor for developing Amyotrophic Lateral Sclerosis (ALS). Previous reports suggest estrogens may be a neuroprotective factor in ALS. Thus, we decided to describe the gender-ratios and disease progression considering, Survival, Forced Vital Capacity (FVC) and Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) like instruments for evaluating the evolution of functional status of male and female patients in different age groups.

Objective: The aim of this study is to describe the differences between gender-ratios and disease progression in males and females along different age groups.

Methods: The study population included every patient diagnosed with suspected, possible, probable or definite ALS (according to the El Escorial Criteria) attending the ALS Unit of the University Hospital "12 de Octubre" since 1 January 2005to 31 April 2012to study Survival Curves and since 1 January 1999/ to 31 April 2012/to determine gender ratios. Patients were divided into two groups: pre or postmenopausal (patients in the peri-menopausal age were excluded) and later distributed in 10 years-age-intervals. The gender ratios were calculated for each group. Peri-menopausal age interval for Spanish women was considered 46.7 to 50.1 years based on statistics published in the Ministry of Health. To study disease progression, patients selected had to have at least 3 clinical evaluations. The events considered for comparison of survival curves were: date of death, $26 >$ ALSFRS < 22 values and $75\% >$ FVC $< 65\%$ values.

Results: 223 individuals were included in the gender ratio study. For the first analysis 48 were excluded (peri-menopausal). The pre and post-menopausal frequencies between males/ females were 29/9 and 144/70. In the second analysis a progressive trend can be observed to increase in th

with a sharp change in the peri-menopausal interval. Patients older than 50.1 years (both men and women) have similar survival curves (almost 100% died at 80 months after the start of symptoms), but in this age group the disease progression is observed to be faster in women. Surprisingly, in patients below 46.7 years, 40% of men surviving at 70 months after the start of symptoms, while 100% of women do not survive. Unfortunately, the sample size to evaluate the disease progression in this age range considering ALSFRS and FVC is very small, and there are no conclusive results.

Discussion: In ALS patients we can observe a decrease in the gender ratio when age at onset is rising as it was possible to observe in incidental studies previously. The differences observed in disease progression (CVF and ALSFRS-r) between genders, should be taken into account in clinical management of ALS patients.

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P142 LEISURE TIME PHYSICAL ACTIVITY IS ASSOCIATED WITH AN INCREASED RISK OF ALS

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Keywords: case-control study, risk factor, physical activity

Background: Ever since Lou Gehrig, a famous professional baseball player, died from amyotrophic lateral sclerosis (ALS), it has been hypothesized that physical activity is a risk factor for developing ALS, fuelled by recent observations that professional soccer players and Gulf War veterans are at increased risk of ALS.

Objective: The aim of the present population-based study was to determine the relation between lifetime physical activity and risk of sporadic ALS, using an objective approach for assessing physical activity in order to minimize recall bias.

Methods: 636 incident ALS patients and 2,166 controls filled in semi-structured questionnaires to obtain lifetime history of occupations, sports, and hobbies. All reported activities were assigned a metabolic equivalent score (MET) based on the Compendium of Physical Activities. The MET is a physiological measure expressing the energy cost of physical activities. Odds ratios for ALS with levels of cumulative occupational and leisure time physical activity were calculated, adjusted for gender, age, level of education, premorbid body mass index (BMI), current alcohol consumption and current smoking.

Results: Higher levels of leisure time physical activity were associated with an increased risk of ALS (adjusted OR 1.08, 95% CI 1.02–1.14). No association with occupational activity or vigorous physical activities, such as marathons and triathlons, was found and the cumulative measures of physical activity in quartiles did not show a dose-response relationship.

Conclusions: We conclude that not increased physical activity per se, but rather a genetic profile and lifestyle promoting physical fitness increase ALS susceptibility.

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P143 EXPRESSION OF HEPATOCYTE GROWTH FACTOR IN THE SKIN OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: immunohistochemistry, hepatocyte growth factor, skin

Background: Hepatocyte growth factor (HGF) is one of the most potent survival-promoting factors for motor neurons, comparable to glial cell line-derived neurotrophic factor *in vitro*. Neurotrophic effects have been demonstrated *in vivo* on embryonic spinal motor neurons during development and on adult motor neurons after axotomy of the hypoglossal nerve. In addition, overexpression of neuronal HGF has been shown to result in the attenuation of neuronal cell death and progression of disease in a familial ALS transgenic mouse model. Therefore, HGF might be beneficial for motor neuron survival. It is unknown, however, whether HGF-positive structures are present in the skin from patients with sporadic ALS.

Objectives: We have carried out an immunohistochemical study of HGF in skin from ALS patients.

Methods: Skin biopsy samples were taken from the upper left arm of 19 patients with ALS (mean age 60.8 years) and from 16 controls with other neurodegenerative diseases matched for sex and age (mean age 59.3 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. The sections were incubated with anti-HGF antibody. After washing in phosphate-buffered saline, biotinylated anti-IgG was applied. The sections were stained by ABC kit. The immunoreactivity was quantified with an image-analysis system. Statistical comparisons were made by the two-tailed Student's t test with $p < 0.05$ as the significance level. Correlation coefficients were calculated by the least-squares method. Results are expressed as the mean ± SD.

Results: HGF immunoreactivity was positive in the epidermis and dermal blood vessels and glands in ALS patients. The HGF-immunopositive (HGF+) structures of the epidermis were the nucleus and the cytoplasm. These findings became more conspicuous as ALS progressed. On the other hand, the epidermis, dermal blood vessels and glands showed a weak HGF immunoreactivity in controls even after repeated antigen retrieval trials. The optical density for HGF immunoreactivity of the nucleus in the epidermal cells in patients with ALS was significantly higher ($p < 0.001$) than in controls. The optical density of the cytoplasm in the epidermal cells in patients with ALS was also significantly higher ($p < 0.001$) than in controls. The density of HGF immunoreactivity in ALS patients showed a progressive increase in relation to duration of illness. This positive correlation was highly significant ($r = 0.53$, $p < 0.02$ and $r = 0.73$, $p < 0.001$, respectively) in the nucleus and the cytoplasm in the epidermal cells.

Conclusions: The conspicuous finding in the skin of ALS was the increased HGF immunoreactivity, which was even more significant with a longer duration of illness. These data suggest that changes of HGF in ALS skin are related to the disease process and that metabolic alterations of HGF may take place in the skin of patients with ALS.

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P144 HAIR MINERAL STATUS IN ALS PATIENTS

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Keywords: hair, minerals, heavy metal

Background: Little is known about hair heavy metal status in ALS patients. This study evaluated the characteristics of hair heavy metal in patients with ALS.

Objectives: The aim of this study is to evaluate the deposition of heavy metal in the hair and to find out the correlation between heavy metal and ALS.

Methods: Thirty five patients diagnosed with ALS according to the El Escorial World Federation of Neurology criteria were enrolled as the case group. Thirty five (25 male, 10 female) with ALS were recruited at the Department of Acupuncture and Moxibustion, Wonkwang Gwangju University Hospital, from November, 2010 to May, 2012, and hair tissue heavy metal including mercury, lead, aluminum, barium, cadmium, arsenic, uranium, bismuth and thallium were analyzed.

Results: For the deposition of mercury, 11(31.4%) out of 35 patients were higher than normal range. 8(22.9%) as to barium and 4 (11.4%) patients as to lead were higher than normal range respectively. And, 2 (5.7%) got higher than normal range each for the aluminum and cadmium.

Discussion and conclusion: ALS patients should be more careful about food containing mercury. Sixteen patients took a tranquilizer containing Barium. That's why the deposition of barium ranked the second. Lead, aluminum and cadmium is not relevant to drugs. So, in our opinion, ALS patients should avoid food containing lead, aluminum and cadmium.

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P145 NEUTRON ACTIVATION ANALYSIS FOR TRACE ELEMENTS IN SCALP HAIR FROM ALS PATIENTS AND RESIDENTS OF THE KII PENINSULA, JAPAN

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Keywords: neutron activation analysis, scalp hair, metals

Background: Oxidative stress has been speculated to play a role in the development of amyotrophic lateral sclerosis (ALS). The Muro district in the Kii Peninsula of Japan has a high incidence of ALS and parkinsonism-dementia complex (PDC). The district drinking water contains severely low levels of Ca and Mg, and the serum of patients and residents in the district showed markedly low levels of Ca and Zn. We speculated that a chronic essential mineral deficiency might induce an accumulation of toxic metals, and consequently increase metal-induced oxidative stress on neurons.

Objective: We investigated metal contents in the scalp hair of patients with ALS or PDC in the Muro district.

Methods: Hair samples (approximately 200 mg) from patients with ALS or PDC in the Muro district, residents in the Muro district and controls were collected and washed with acetone, distilled water and acetone in sequence and then dried in air. Approximately 30 mg of the sample was weighed and double-wrapped in polyethylene films and subjected to neutron activation analysis at Kyoto University Research Reactor (KUR). The samples in polyethylene capsules were irradiated in Pn-1 of KUR (1000 kW) for 2 minutes and for 120 minutes, for short and long irradiations, respectively. As comparative standards, orchard leaves (NBS) as well as elemental standards for Ca, Mg, V and Zn were used. The gamma-ray spectroscopic measurements with a Ge detector were performed repeatedly. For the short-lived nuclides, the measurement time was 200 seconds after a cooling time of 110–320 seconds. For the long-lived nuclides, the measurement time was 5000–6000 seconds after cooling for approximate one month.

Results: Six patients with ALS and five patients with PDC from the Muro district, 66 residents from the Muro district and 22 controls participated in this research. Contents of Ca, Mg, Al, Cu, Mn, Cl, Hg, V, S and Zn were determined in the hair samples. To avoid influences by perm and dyeing, data in subjects without chemical procedure were compared among these groups. The Mg and V contents from patients with ALS (1107.02 ± 1068.15 ppm, 168.49 ± 298.16 ppb, mean $\pm$ S.D.) and the Mg contents of patients with PDC (855.68 ± 200.45 ppm) were significantly higher than those of controls (224.31 ± 135.06 ppm, 19.57 ± 13.79 ppb, $p < 0.05$, respectively). The Mg contents from residents in the Muro district (366.5 ± 140.32) were also higher than those of controls (224.31 ± 135.06, $p < 0.05$).

Discussion and conclusions: It is known that excess of V induces oxidative stress on neurons and Mg contents in hair samples increase in secondary hyperparathyroidism. The present results indicate an imbalance of metal metabolism in patients with ALS or PDC in the Muro district.

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P146 ALS IN THE FRENCH ALPS AND THE CYANOBACTERIAL NEUROTOXIN BMAA HYPOTHESIS: FIRST CASE WITH BMAA POSITIVE BRAIN REPORTED IN EUROPE

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Keywords: epidemiology, cluster, BMAA

Objective: Amyotrophic lateral sclerosis (ALS) clusters are a chance to underline environmental risk factors

the disease. We depicted ALS clusters in French Alps (Savoy) and present results of BMAA analysis within the brain of the first case autopsied, coming from one cluster identified.

Background: The existence of environmental risk factors for ALS has been underlined by spatial clusters and hyper-endemic zones such as Guam or Kii. In these later areas, it has been strongly suggested that the neurotoxin BMAA could be one/the etiologic risk factor, due to its presence in the brain of ALS cases, secondary to its biomagnification in diet.

Design and methods: We identified Savoy cases from 2002–2010 with a multi-source system (Grenoble teaching hospital and data from the different insurances systems), and calculated Standardized Incidence Ratio (SIR) for each administrative district. For one of the highlighted clusters, we sent brain autopsy tissue of one deceased patient to the partner team of the Institute of Ethnomedicine to search for BMAA. Hydrolysates of this tissue underwent solid phase extraction to purify amino acids and AQC derivatization before BMAA analysis using liquid chromatography triple quadrupole mass spectrometry.

Results: Clusters of sporadic ALS were detected in two villages of Savoy among patients who were not relatives (SIR = 24.6(4.9–71.8), SIR = 14.0(2.8–40.8)). One living patient agreed for a post mortem autopsy. BMAA was positively identified in the frontal temporal lobe of the brain based on four mass spectrometry criteria in comparison with an AQC derivatized authenticated BMAA standard. Among the possible risk factors that could be associated with biomagnification, this patient was eating local mushrooms, and cyanobacteria were detected on the moss where they grow (phycocyanin detection with fluorimetric probe).

Conclusion: This case raises the question of the relevance of the BMAA hypothesis for some ALS cases in Europe, a hypothesis that will be tested to a larger extend in France.

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P147 WAS DRIED FISH A TRIGGER OF HIGH-INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN KII PENINSULA, JAPAN?

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Keywords: nutritional factor, epidemiology, population-based study

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, which has been linked to the generation of free radicals and oxidative stress. The Koza, Kozagawa and Kushimoto area in the Kii Peninsula of Japan (K area) is a well-known high incidence area of ALS with low calcium and magnesium contents in soil and drinking water sourced from Kozagawa River in the K area. Although it is obviously predicted that those status is affected by nutritional status, few studies have examined nutritional states in population living in K area.

Objective: The aim of this study was to explore nutritional factors to look for the explanatory clues of the high incidence of ALS in the K area from comparisons with the area free from ALS (control area) together with the results of a population-based case-control study conducted in Japan.

Method: A cross-sectional population-based survey was conducted for adults aged ≥ 15 years living in K area and control area in 2003 and 2010. A case-control study was conducted with incident 183 ALS cases and 407 controls. Dietary information was obtained by a self-administered food frequency questionnaire (FFQ), consisting of 97 commonly eaten food and beverage items. Frequency of food consumption was classified into two categories as follows; low ("never/seldom" or "less than once a week" "1–3 days a week"), and high ("4–5 days a week" or "almost everyday"). The differences of means were tested with analysis of variance and of the distribution of proportions with the chi-square test.

Results: In 2003 and 2010, K area had a significantly lower percentage intake of fat and a higher percentage intake of protein; a proportion of the fish consumption to the total amount of consumption and lower than that of meat than the control area. Nutrient adequacy of fish was the highest among that of food consumption in both 2003 and 2010. Among various kinds of seafood, the percentage of subjects with high frequency of dried fish consumption was significantly higher in K area than in control area, but not that of fish and shellfish consumption. In this case-control study, habitual high frequency of dried fish consumption was significantly associated with an increased risk of ALS, even after adjusting for confounding factors (low vs. high, adjusted OR: 4.88 (95%CI; 3.60–10.3).

Conclusion: The present results suggested that habitual high frequency of dried fish consumption might have triggered the development of ALS in the K area, especially considering that our case-control study showed a positive association between habitual high frequency of dried fish consumption and the ALS risk.

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P148 CROSS-SECTIONAL SURVEY OF VISUAL ACUITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: vision, biomarker, prevalence

Background: Clinical and post-mortem observations of pathological changes outside the motor system in some people with amyotrophic lateral sclerosis (ALS) have led to a shift from the classical characterization of ALS as a disease exclusively of motor neurons to that of a multisystem disorder. Visual system disorders are a recent addition to the phenotypic spectrum of ALS based on a study that showed decreased binocular visual acuity (VA) in ALS patients (1). We hypothesize that decreased VA in ALS patients is due to neurodegeneration of the visual pathway. Monocular VA and binocular summation (improvement in VA when tested with two eyes compared with one eye) measurements can guide localization of visual pathway dysfunction. These have not been previously characterized in ALS patients.

Objective: To determine if monocular VA and binocular summation are reduced in ALS patients compared with control subjects.

Methods: Patients attending a multidisciplinary ALS clinic (n = 15, age 48.4 + /−3.1 years) and their caregivers, serving as controls (n = 8, age 48.5 + /−3.1 years), participated in this study. Monocular and binocular VA were assessed using high (100%) and low (2.5%, 1.25%) contrast charts (Sloan) under controlled lighting conditions following refraction. Binocular summation was calculated as the difference between binocular and best monocular acuity scores.

Results: Low contrast VA was reduced by approximately 1 line in ALS patients versus control subjects in both binocular (OR 0.79 (0.62–0.98), p = 0.032, logistic regression accounting for age) and monocular (OR 0.78 (0.59–0.97), p = 0.029, logistic regression accounting for age) conditions. High contrast VA and high and low contrast binocular summation were not different between groups.

Discussion and conclusions: We confirm that binocular VA is decreased in ALS patients and also find this to be true for monocular testing. Binocular summation is not affected. This supports a hypothesis of visual pathway pathology anterior to the lateral geniculate nucleus as the etiology of decreased visual acuity in ALS patients. Further study is warranted regarding the pathophysiologic basis for these results and their application to disease characterization and outcomes assessment.

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P149 ALOPECIA AND AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: alopecia, epidemiology, androgen receptor

Background: A recent study showed that a low index-to-ring (2D: 4D) finger ratio was associated with amyotrophic lateral sclerosis (ALS), suggesting that prenatal exposure to testosterone or an increased sensitivity of androgen receptors (AR) may play a role in ALS development. Increased sensitivity of AR causes early onset alopecia, however it is unknown if alopecia is associated with ALS risk.

Objectives: To explore the association of early onset alopecia and ALS in a prospective cohort.

Methods: We explored the association of early onset alopecia and ALS using the Health Professionals Follow-up Study (HPFS), consisting of 51,529 men aged 40–75. HPFS was established in 1986 and has been updated biennially since then. In 1992, participants were asked to report their hair line pattern at age 45 using five pictures of different hair line patterns. The National Death Index was used to identify ALS deaths. In addition, incident ALS was self-reported every two years starting in 2000, and confirmed based on information from medical records or the treating neurologist. Uncertain ALS diagnoses were later included if confirmed through the National Death Index. Cox proportional hazards models were used and we adjusted for age in single years, body mass index, physical activity, smoking and intake of vitamin E. There were 42 participants diagnosed with ALS during follow-up (1992–2008).

Results: Of the 42 participants diagnosed with ALS during follow-up, 13 reported no alopecia at age 45, 18 reported some alopecia and 11 reported extensive alopecia at age 45. Participants reporting extensive alopecia had a 179% increased risk of ALS compared with those reporting no alopecia (RR 2.79; 95% CI 1.25–6.23). Further, we observed a linear trend of increased risk of ALS with increasing level of balding at age 45 ($P_{trend} = 0.02$).

Discussion and conclusion: The results of this prospective study are consistent with the involvement of AR in the etiology of ALS, a hypothesis proposed in 1980 based on the observation that ALS affects all motor neurons and cranial nerves except cranial nerves III, IV and VI that also lack AR. The cause of alopecia is not fully understood, but is likely polygenetic. A GWAS on 95 families with early onset alopecia revealed 14 chromosomal regions linked to alopecia. Five of these regions overlap known ALS genes, including SOD1, OPTN and ANG. The association of alopecia and ALS, as well as AR and ALS should be further explored in other populations.

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P150 FLEXIBLE COMMON DATA STRUCTURE METHODS AND PLATFORM ALLOW FOR POST-HOC CUSTOMIZATION

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Keywords: common data elements, pooled resources, collaboration

Background: To develop disease progression models and identify disease markers it is essential to have access to large datasets merged from multiple clinical trials. The Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform utilizes novel data mapping methods allowing users to homogenize multiple sets of data.

Objectives: To utilize methods and algorithms for creation and management of a flexible Common Data Structure (CDS) in ALS that allows mapping heterogeneous datasets from ALS clinical trials and changing the CDS, if necessary, without data re-importation.

Methods: Data coming from a multitude of sources must be homogenized and organized according to a CDS for which the following was performed:

- CDS was created to accommodate NINDS-recommended and NEALS Common Data Elements (CDE)
- Source data were analyzed and, if not provided, a data dictionary was created using the column names and possible values contained in each column
- Relationships were identified between the source datasets and the CDS
- Based on these relationships, a map was created linking each source data field to a CDS data field
- The data were de-identified by removing any information that could connect the entered data to a specific subject or study (date of birth, date of collection, initials, etc.)

- The source datasets were imported into the PRO-ACT platform preserving the natural groupings and properties
- During importation, the system generated unique subject ID's to further de-identify each data point.

Results: The PRO-ACT platform was designed to enable users to create highly-customizable disease-specific CDS. In addition to being flexible, this design allows the CDS to be modified, without the need to re-import existing data. There are currently over 900 CDEs in the PRO-ACT Common Data Structure that are being used to map data from eleven ALS clinical trials donated by three pharmaceutical companies in addition to six NEALS trials. These CDE were specified while adhering to NINDS and NEALS Common Data Elements guidelines wherever possible.

Conclusions: The deployed PRO-ACT platform enables users to build disease-specific CDS that allow heterogeneous datasets from multiple clinical trials to be mapped and imported to create a single homogenous dataset. If there is a need to change the CDS because of new guidelines and discoveries, the platform allows users to execute the change without data re-importation.

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P151 INFRASTRUCTURE RESOURCES FOR CLINICAL RESEARCH IN ALS

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Keywords: collaboration, resources, biobanks

Background: Clinical trials networks, shared clinical databases, and human biospecimen repositories are examples of infrastructure resources aimed at enhancing and expediting clinical and/or patient-oriented research to uncover the etiology and pathogenesis of amyotrophic lateral sclerosis (ALS).

Objectives: To compile and analyze information on available international infrastructure resources for ALS research and to discuss current impediments, gaps and opportunities.

Methods: A survey on current infrastructure resources was sent to 102 specialized ALS Clinics in North America. Analysis of 57 responses has revealed an established infrastructure of the following resources:

- Clinical databases: Individual sites–44 (77%; sites as a consortia member 20 (35%)
- Biospecimen collections including Plasma, CSF and DNA/RNA: 23 sites (40%) and skin biopsy: 14 sites (25%)
- Tissue and autopsy programs including muscle biopsy: 29 sites (51%) and brain banking: 19 sites (33%).

The "Second Tarrytown ALS Meeting" held in September of 2011 had a separate session on international ALS infrastructure resources developed and maintained by ALS Clinics and Centers in North America and Europe, various clinical trial networks, US government federal agencies including the National Institutes of Health (NIH), and several voluntary disease organizations that support ALS research activities. European biobanks and registries include the UK National Motor Neurone Disease DNA Biobank, the South East England ALS Registry (SEALS) registry; and a pan-European FP-7 grant that coordinates data and biospecimen collections in five EU countries.

Results: Many specialized ALS clinics in North America developed site-specific infrastructure resources for patient care and clinical research. They rely on a variety of funding sources, including US government agencies, national and local patient support organizations and philanthropic groups. The development of multi-clinic datasets may facilitate and enhance clinical research. This will also enable more seamless communication among clinical research centers. Challenges include the requirement for dedicated financial support, standardization of collected data elements, consistency in data capture, and the need to overcome possible psychological barriers to sharing de-identified clinical information among individual clinics.

Conclusions: To encourage collaboration and to optimize utilization of resources in ALS research, the following is paramount: 1) establishment of shared databases among individual ALS Clinics to enhance the coordination of resources and data analyses; 2) expansion of quality-controlled human biospecimen banks; 3) adoption of uniform data standards, such as Common Data Elements (CDEs) for ALS clinical research and clinical practice.

The value of clinical trials networks such as the Northeast ALS (NEALS) Consortium, the Western ALS (WALS) Consortium and European ALS Consortium shall be recognized and collaboration and coordination between them shall be encouraged, including development of strategies to further enhance and complement these networks and their research resources.

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THEME 7 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P152 COST ANALYSIS OF MULTIDISCIPLINARY ALS CLINICS IN THE VETERANS HEALTH ADMINISTRATION

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Keywords: cost, multidisciplinary clinics, veterans health administration

Background: In a 2003 study regarding multidisciplinary amyotrophic lateral sclerosis (ALS) clinics Traynor and colleagues (1) showed that these clinics increase survival and decrease mortality when compared to general neurology clinics. However, the cost of these clinics has not been extensively studied. The Veterans Health Administration (VHA) is a unique system in that patients receive all their care within a single payer system, allowing for reliable cost analysis.

Objective: To determine if there is a cost difference between multidisciplinary and general ALS clinics within the VHA.

Methods: Staff at the Office of Productivity, Efficiency and Safety (OPES) used the Corporation Data Warehouse to obtain Decision Support System National Data Extract datasets for FY2010. Data merging and analysis were done through scrambled SSN; no patient identifiable information such as name, SSN or addresses were used or revealed. Data was obtained according to patient utilization across different facilities. ALS patients were identified by ICD-9 codes from the inpatient, outpatient and fee files. Multidisciplinary ALS clinics were classified as care offered by a Neurologist or Physiatrist and at least 2 other service lines (PT, nutrition, etc.) at the same visit. All other clinics, including Neuromuscular Clinics, were labeled 'general clinic'. Medical centers with more than 20 ALS patients were identified from a total of 139 facilities within the VHA. 68 medical centers fit this criterion. All 68 medical centers were contacted by phone to obtain information regarding the presence or absence of a multidisciplinary ALS clinic. Nine medical centers were excluded as accurate data could not be obtained and one medical center was excluded due to extremely large unexplained variations in cost. Twelve medical centers were identified as having multidisciplinary ALS clinics. ANOVA testing was done to determine statistical significance.

Results: Total cost of ALS care per patient for FY2010 was $41,186 in general clinics versus $42,089 in multidisciplinary clinics ($p < 0.05$). The average inpatient, outpatient, and fee expenditure costs per patient were $15,063, $19,260, and $6,863 in general clinics versus $15,575, $22,280 and $4,234 in multidisciplinary clinics. Inpatient costs were 37% of the total cost in both clinics. Outpatient costs were 6% higher in the multidisciplinary clinics, which were offset by 6% higher fee basis costs in the general clinics.

Discussion and conclusions: Our study shows that there is no statistically significant difference in the cost of ALS care provided in multidisciplinary versus general clinics in the VHA. Taken in conjunction with previous studies that have shown increased survival and decreased mortality in multidisciplinary clinics, these results strengthen the case for providing patient-care in multidisciplinary ALS clinics.

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P153 VARIATIONS IN COST OF ALS CARE WITHIN THE VETERANS HEALTH ADMINISTRATION

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Keywords: variations, veteran health administration

Background: The Veterans Health Administration (VHA) allows for reliable cost analysis as patients receive all their care within a single payer system. Amyotrophic lateral sclerosis (ALS) is a fully compensable illness within this system making cost studies related to the disease ideal. Although there have been studies evaluating the cost of ALS care, there have not been many looking at variations in costs based on facility location, academic affiliation and physicians in training.

Objectives: To ascertain the cost of ALS care within the VHA for fiscal years 2005–2010. To analyze the cost of care in rural versus non-rural facilities, teaching versus non-teaching facilities and those facilities with neurology residents versus those without neurology residents.

Methods: Staff at the Office of Productivity, Efficiency and Safety (OPES) used the Corporation Data Warehouse to obtain Decision Support System National Data Extract datasets for fiscal years 2005–2010. Data merging and analysis were done through scrambled social security numbers; no patient identifiable information was used or revealed to the investigators. ALS patients were identified by ICD-9 codes from the inpatient, outpatient and fee files (care provided outside the system but paid by VHA). ANOVA testing was done to compare rural versus non-rural facilities, teaching versus non-teaching facilities, and those facilities with and without Neurology residents.

Results: Total cost of ALS care between FY2005-FY2007 ranged from $35,890,466 to $37,389,552. The cost increased to $47,805,567 in FY2008, $81,802,784 in FY2009, and $114,288,333 in FY2010. The number of ALS patients between FY2005 and FY2008 ranged from 1488 to 1599. The number increased to 2304 patients in FY2009 and 2752 patients in FY2010. Total cost per patient increased from

$23,581 in FY2005 to $41,529 in FY2010. The cost per patient in rural versus non-rural ($46,969 versus $40,561), teaching versus non-teaching ($43,370 versus $46,419) and neurology residents versus no neurology residents ($39,509 versus $48,450) only showed statistical significance (p-value < 0.05) in the latter.

Discussion and conclusions: The cost of ALS care has more than tripled in the VHA between fiscal years 2005 and 2010. A large portion of this increase occurred after 2008 when ALS became a compensable illness. The total cost per patient has also increased between 2005 and 2010, which is likely due to increased utilization of services offered by the VHA to veterans as well as increasing costs of providing care. The cost of inpatient, outpatient and fee expenditure care have all increased in a proportional manner. The cost per patient of outpatient care is 20% higher than the cost of inpatient care. There is no statistically significant difference in cost between rural and non-rural facilities as well as teaching versus non-teaching facilities. There is a statistically significant higher cost of ALS care per patient in those facilities with Neurology residents.

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P154 COST ASSESSMENT OF MULTIDISCIPLINARY CLINICS ADHERING TO AAN ALS PRACTICE STANDARDS

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Keywords: multidisciplinary, costs, standards

Background: The multidisciplinary team approach to the treatment and management of amyotrophic lateral sclerosis (ALS) patients is the preferred model of care, with evidence for prolonged survival, improved quality of life, and reduced hospital admissions. (1,2,3,4) There are no published data on costs associated with this model of patient care for U.S. institutions that adhere to the American Academy of Neurology Practice Parameters (AANPP).

Objective: To address these issues, in 2007 five centers providing multidisciplinary care participated in a retrospective review of clinic costs using a self reported data collection tool over a six-month period.

Methods: Each of the five centers self-reported patient volume, staff time, salary and benefit costs; non-clinic staff time; costs of supplies, equipment and overhead including rent, departmental fees and institutional fees at their clinic. All centers met criteria established for ALS Association certified multidisciplinary clinics. Results were presented in a descriptive manner.

Results: A total of 502 patients were seen during the six-month study period, range 35 to 220 patients per center. The total annualized patient visits was 1,645. Non-salary direct and indirect expenses accounted for 3–14% of total expenses at four centers. Annualized staff salaries and benefits were $1,016,405. The annualized mean non-salary direct and indirect cost was $42,000, and mean annualized total expense was $245,000 per center. Mean total expense per clinic day was $5,800 and mean expense per patient per clinic was $772. The mean annual expense per patient was $2,737.

Discussion and conclusions: Results from this pilot study provide an initial estimate of U.S. clinic costs and are useful in identifying the elements of cost of multidisciplinary care conforming to the AANPP.

Investigators decided to expand pilot data and obtain information from a larger number of diverse centers (geography, size, and institution type) so as to garner more reliable and generalizable data. A new and expanded study is employing a prospective approach and also includes an estimate of clinical outcome measures to evaluate practice standards and patient satisfaction. Already underway, this three-month prospective descriptive, multi-center study examines costs associated with care provided at 15 U.S. geographically diverse sites providing multidisciplinary ALS care according to the AANPP. Initial data from the expanded study will be available for presentation at the Symposium.

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P155 HEALTH SERVICE USE BY PEOPLE WITH ALS IN SOUTH EAST ENGLAND

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Keywords: health service use, equipment, symptom severity

Background: Research has begun to provide data on health service and equipment use by people with amyotrophic lateral sclerosis (ALS) in the US, Scotland, Southern Ireland and Spain. Studies have shown that health resource use increases progressively with disease severity and that a wide range of equipment is needed irrespective of disease onset site.

Objectives: As part of a larger prospective, population-based study investigating decision-making about interventions in ALS, our objective was to measure longitudinal health service use by patients in South East England. Here we report our sample's service use at study recruitment (bas

Methods: People with ALS were recruited from the South East ALS Register between six and sixty months post-diagnosis. At baseline, participants completed various physical, cognitive and psychological measures. Seventy-five participants also completed a questionnaire about their use of inpatient, outpatient and community services over the preceding three months, and the informal help they received during this time. They were also asked about any equipment obtained and adaptations made to their home. Participants updated this information every three months until death or till the end of the study.

Results: The sample had a mean age of 62.5 years (SD 11.7), and had been diagnosed on average 12.5 months prior to recruitment. Forty-nine were male. The majority had sporadic ALS (71) and non-bulbar onset (65). The mean ALSFRS-R score was 35.3 (SD 7.5). At baseline, very few patients had used inpatient services. Outpatient services were more frequently used, with the majority (56%) seeing a neurologist within the preceding three months and 21% attending a hospice day centre. The most commonly reported community service was the GP (72%). Relatively high numbers accessed occupational therapy (68%) and physiotherapy (63%). Speech therapy (SLT) input was less common (45%). Analysis of ALSFRS-R scores of those using/not using services revealed the largest differences in SLT, personal care and palliative care nursing, with users having greater symptom severity. SLT use differed according to region of onset and ALSFRS-R speech/swallowing scores. Walking aids and manual wheelchairs (51%) were the most commonly used equipment types. Use of the latter was more common in those with lower ALSFRS-R scores. Few patients had major adaptations to their home. The amount of informal help received ranged from none to constant help (mean 20.2 hours/week).

Discussion: This study characterizes current health service use, equipment and informal help received by people with ALS recruited from the South-East ALS Register on average 12.5 months post-diagnosis. Symptom severity was meaningfully related to use of some services, and equipment and amount of informal help required, and service use was related to individual needs. Further publications will plot change in service use over time and examine relationships between service use and intervention uptake.

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P156 IMPORTANCE OF SOCIAL WORK IN DEVELOPING PERFORMANCE MEASURES FOR ASSESSING CARE FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: advanced directives, clinic audit, benchmarking

Background: Social work plays a crucial role in the care of patients with amyotrophic lateral sclerosis (ALS). Social work is important in helping patients cope with the rendering of the diagnosis, learn about resources for implementing therapy, dealing with disability, and education and support for the end of life process.

Objective: To identify the role of social work in increasing the proportion of patients who have advanced directives in place and increasing the deployment of appropriate hospice utilization among patients as the disease progresses.

Methods: Social work involvement begins at the first clinic visit when patients who may have ALS are being diagnosed. The initial indepth interview is followed by regular follow up sessions to complete advanced directives between regular clinic followup visits. At the ALS Multidisciplinary Clinic or ALS Ventilator Clinic followup visits social work intervention is optional but nearly universal. Social work involvement is needed for access to care, financial support for treatments and home care, interactions with caregivers, involvement in end of life planning and access to hospice care. Performance measures evaluated for social work contributions to overall ALS care included proportion of patients completing advanced directives and proportion of patients receiving appropriate hospice care at end of life. Statistical analysis was performed with MedCalc Software (Leuven, Belgium).

Results: Surprisingly the proportion of patients completing advanced directives was not 100%. In various groups 72.5 ± 6.4 % of patients completed advanced directives following the first visit. In veterans with ALS who are served at our ALS clinics and who receive care at Department of Veterans Affairs, it was expected that the proportion of patients completing advanced directives would be 100% since that is a major feature of care in that hospital system, however, the proportion of veterans completing advanced directives was similar to the general population. The time from first visit to completion of advanced directives was 9.2 months (95%CI = 4.4–18.6 months). Involvement in hospice care was not universal in our clinics. More patients above 65 years were involved for a larger time than those patients under 65 years.

Conclusions: Social work performance measures identify areas for improvement in ALS care with needs to achieve higher compliance with respect to completing advanced directives and increasing the proportion of younger ALS patients who employ hospice support at the end of life.

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P157 WEEKLY AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENT MANAGEMENT CONFERENCE–PIVOTAL ROLE IN PATIENT CARE QUALITY IMPROVEMENT, EDUCATIONAL AND COMMUNITY OUTREACH OF CAROLINAS NEUROMUSCULAR/ALS-MDA CENTER DISEASE-SPECIFIC CARE CERTIFICATION PROCESS

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Keywords: disease-specific certification, benchmarking, multidisciplinary

Background: Crucial to evidence-based guideline management of the care of patients with amyotrophic lateral sclerosis (ALS) is a structure to implement at a per-patient level adherence to these guidelines. Lessons from other diseases, including cancer and cardiovascular disease, indicate that regular review of the diagnosis, initial and follow-up treatment decisions, treatment deployment coupled with acceptance of and compliance with treatment are efficiently managed with a multidisciplinary clinic model. Patient adherence to treatment over the course of disease and changes in recommended treatment is aided by regular review at a per-patient level. The regular audit of patient care has been shown to increase timeliness of proper diagnosis and treatment.

Objective: To describe the structure and function of the Weekly ALS Patient Management Conference at the Carolinas Neuromuscular/ALS-MDA Center in the Department of Neurology at the Carolinas Medical Center in the Carolinas Healthcare System in North Carolina and South Carolina.

Methods: Patients from the Piedmont and western mountain regions of both North Carolina and South Carolina with uncertain, as well as established, diagnoses attend the weekly ALS Intake Clinics for a two-day multidisciplinary clinic evaluation. Each new patient's clinical summary is presented at the Weekly ALS Patient Management Conference by the MD, allowing input from each member of the multidisciplinary clinic team. Treatment plans are formulated and any limitations to initiating these plans are identified by the RN Clinic coordinator/staff. Each week, active patient problems identified by telephone, email or direct contact are reviewed with formulation of potential solutions. Patients with clinical changes that warrant home visits are reviewed by the RN Homecare coordinator. Patients who attend the monthly one-day Ventilator Clinic for patients with tracheostomy and permanent ventilation and who attend the monthly two-day Multidisciplinary Clinic for patients with and without non-invasive ventilation are discussed at the weekly conference prioritized by number of new problems to be addressed. Patient Safety events during clinic, emergency department visits with and without hospitalization, palliative care, hospice and end-of-life referrals and deaths are reviewed. Minutes are kept in rotation by a staff member and circulated by secure email to staff members/allied health/other consultants who participate in the diverse clinic programs but who are not at the weekly conference.

Results: In the period 2010–2011, 170 new patients were reviewed, of whom 12 had diagnoses other than ALS. Ventilator clinic (31) and Multidisciplinary clinic (204) unique patients provided 124 and 816 encounter discussions. Home visits generated 398 discussions and patient problems generated 832 discussions. There were 8 patient clinic fall-related safety discussions. Deaths(62), hospice referrals (56) and hospitalizations (19) were reviewed.

Conclusions: Weekly ALS Patient Management Conference provides mechanism for 1) per-patient diagnosis, treatment plan review, 2) patient clinic review, 3) patient disease milestone review and 4) patient co-morbidity review.

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P158 INTEGRATION OF HOME CARE INTO DISEASE-SPECIFIC CERTIFICATION BY HOME CARE RN COORDINATOR AT CAROLINAS NEUROMUSCULAR/ALS-MDA CENTER: DEVELOPMENT OF BENCHMARKS FOR IMPROVING PATIENT CARE

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Keywords: home care, disease-specific certification, benchmarking

Background: Home care nurse visits are employed to provide care as well as monitor care given at home to patients with ALS. Home care may be divided by who provides the care: caregiver, non-hospice-care or hospice-care (1). The goal of the Carolinas Neuromuscular ALS-MDA Center RN Home Care Coordinator is to facilitate guideline-based care in the home setting for patients with ALS and in nursing homes when patients are cared for in that setting.

Objective: To identify the elements of guideline-based care monitored in the Carolinas Neuromuscular ALS-MDA Center RN Home Care program.

Methods: Home visits occurred per protocol by the Home RN Review of 418 home visits (48% M; 52% F) from 2010–2011 at weekly ALS Patient Review conference to identify criteria for home visits and evaluate adherence to guideline-based care as part of an on-going program to develop performance measures for disease-specific certification of care for patients with ALS

Results: Home visits occurred following gastrostomy, tracheostomy, recent hospitalization or emergency department visit to assess medication and equipment compliance as well as safety review to prevent falls at home. The mean time to first home visit was 8.6 ± 9.4 (standard deviation) months following first clinic visit. Patients received 1–5 visits (mean

3.1 ± 2.8) Patients had lower mean ALSFRS-R scores (18.2 ± 20.3).Some patients who initiated non-invasive ventilation were seen following respiratory therapy home visits when compliance issues were identified. Home visits included monitoring wound care, assistive speech technology, and initiation of home hospice and ALS care education for patients in nursing homes.

Conclusions: Home visits occurred within one year following the first clinic visit. Home visits identifying deviations from guideline-based care were discussed at the Weekly ALS Patient Care Conference to develop potential interventions. Rates of deviation form guideline-based care provide benchmarks for future interventions to improve care at home.

Discussion: Home visits provide a means to enhance compliance to guideline-based care between clinic visits (2, 3).

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P159 CURRENT STATUS OF COORDINATORS FOR PATIENTS WITH INTRACTABLE DISEASES IN JAPAN

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Keywords: intractable disease, co-ordinator

Background: Co-ordinators for patients with intractable diseases (Co) are assigned to manage and supervise circumstance for each patient in most of the prefectures in Japan. They have been acting with multiple health professionals and have been supposed to seek further facilities not only for respite care, but also for long-term services. In addition, Co is involved in a wide range of activities for providing information, running a workshop for health professionals, and doing research. However, there are considerable differences in work content, professional situation and condition for employment among prefectures. Such background may have made Co feel uneasy to maintain and improve expertise of their work.

Objective: To disclose current status of Co including their tasks, skills, intelligence, and behavioral pattern and propose what is needed for Co in Japan.

Methods: Subjects were Co or substitutes in charge of intractable diseases in 47 prefectural governments. We sent a questionnaire for professional situation, cooperation with other health professionals, tasks for education, utilization of a guidebook made by Co, achievement of the activity, work contents and condition for employment.

Results: Fifty five Co were assigned in 39 prefectures (83% of Japanese prefectures) in February 2012. The collection rate was 82% retrieved from Co and 74% from prefectures. Co's professional backgrounds were mostly nurses (39 were qualified nurses, which included public health nurses, and 6 were medical social workers). Condition for employments as Co was full-time in 29 and part-time in 16. Mean Co tenure was 47.5 months. Fourteen (30%) had been working as Co less than 17 months. Thirty one (68%) were assigned as only one Co in each prefecture. Twenty nine (64%) had reported no opportunity of training as a co-ordinator before becoming Co. After becoming it, 42 (93%) had participated in some workshops, and 37 (82%) attend to scientific meetings in a year. Their self-job-evaluations were relatively good about consultation and cooperation with other health professionals, although they estimated lower on finding facilities for long-term service, respite care, or disaster contingency planning. Co and prefectural governments had a similar view on workloads under very low budgets.

Conclusion: Co in Japan have limited opportunity for training and quite a few have a relatively short tenure. Co's activities of finding facilities for long term and respite services are found to be still insufficient in Japan.

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P160 GUIDELINES ON NEUROLOGICAL PALLIATIVE CARE

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Keywords: guidelines, palliative care, neurology

Background: The European Association for Palliative Care and the European Federation of Neurological Societies have collaborated to produce guidelines on the palliative care of neurological disease, including ALS/MND. A taskforce of the EAPC has developed these guidelines and the authors are presenting the results on behalf of taskforce.

Objectives: These guidelines are aimed at encouraging collaborative working between neurology and specialist palliative care services to improve the care of people with ALS/MND

Methods: The guidelines have been developed from a literature search of relevant articles and using a group of experienced clinicians and researchers in both palliative care and neurology to determine areas for the guidelines agree relevant supporting evidence for recommendations and grade the evidence using the GRADE system.

Results: The following areas of recommendations were made and will be presented with supporting evidence: Palliative care should be considered early in the disease trajectory; assessment of care should be by a multidisciplinary

team; patients should have access to a palliative care assessment; communication with patients and families should be open, including setting of goals and therapy options; early advance care planning is recommended; recognition of deterioration over the last weeks and months is relevant for appropriate management; proactive assessment of physical and psycho-social issues reduces the need for crisis intervention; the principles of symptom management should be applied to neurology; diagnosis of the dying phase allows appropriate management and standardised tools, such as the Liverpool care of the dying patients may be helpful; carers needs should be assessed regularly and support provided before and after death; professionals should receive support and supervision; palliative care principles should be provided in the training and education of neurologists; the understanding and management of neurological symptoms should be provided for specialist palliative care professionals.

Discussion: The development of the guidelines has allowed recommendations on practice for the care of people with neurological disease, including ALS/MND. These will be disseminated to both neurology and palliative care clinicians with the aim of improving the care and quality of life of these patients and their families.

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P161 PRELIMINARY EVALUATION OF END-OF-LIFE CARE FOR PATIENTS SUFFERING FROM MOTOR NEURON DISEASE IN DENMARK

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Keywords: palliative and end-of-life care, place of death, support to relatives

Background: In Denmark, 130 patients are diagnosed with MND annually. Of these, 90–95% are referred to the Danish Rehabilitation Centre for Neuromuscular Diseases (RCfM). RCfM is a specialized centre of excellence for Neuromuscular Disorders. RCfM functions as a key player in the co-ordination of a joint care effort among patients, hospitals and local professionals, and assist affected families with guidance throughout the entire course of the disease. In spite of the specialized care, there is still very little knowledge about the terminal phase and end-of-life care for MND patients in Denmark.

Objectives: To examine the following during the terminal phase: Where do MND patients die? Was the time of death expected by the family and relatives? How many patients use PEG tube, non invasive ventilation (NIV) or invasive ventilation (IV)? Is morphine used? How satisfied are the relatives with the support offered?

Methods: Information was gathered through telephone interviews with the closest relatives or the health professionals, who answered questions about the death from a structured questionnaire.

Results: During the period 1 February 2011–31 January 2012, 120 MND patients died at a mean age of 68 years (range 43–97 years). 65% of them were married, 29% were single, and 6% was unknown. 53% of the replies came from relatives, 41% from health professionals and 6% were not available. 38% died in their own home, 30% at a hospital, 17% in a nursing home, 7% at a hospice, and the place of death for 10% was unknown. 49% died in a desired place, 8% in a non-desired place and for the remaining 43% the answer was not available. From the point of view of the relatives, 54% died at the expected time, 28% at an unexpected time and for 12% the answer was not available. At the time of death, 60% had a PEG tube and in terms of respiratory care, 16% died using NIV whereas 13% were on IV. During the terminal phase, morphine was prescribed for palliative treatment in 50% of cases.

Discussion and conclusion: The terminal phase is difficult to handle without professional help and guidance, and it is crucial to have a well-structured care plan. Insecurity and anxiety are common feelings, and even if the patient wants to die at home, he/she only succeeds in some 50% of the cases. In Denmark only few (7%) are in a hospice during the terminal phase. According to their relatives, approximately one third of the patients died unexpectedly.

Among the Danish MND population, two thirds had a PEG tube and almost one third uses respiratory aids such as NIV/IV. Data from the study of satisfaction with end-of-life care among relatives will be presented.

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P162 BREAKING BAD NEWS IN ALS: THE NEED FOR MEDICAL EDUCATION

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Keywords: medical education, breaking bad news, diagnosis delivery

Background: The manner in which physicians break bad news has been identified as an area of discontent for patients with amyotrophic lateral sclerosis (ALS).(1,2) The American Academy of Neurology's Practice Parameter for care of the ALS Patient identified the following research recommendation: "include disclosure techniques in medical curricula and to evaluate their implementation."

Objectives: To explore the need for medical education as it pertains to residents' ability to break bad news when communicating the diagnosis of ALS.

Methods: Residents were videotaped and graded by two ALS neurologists as they broke the bad news of an ALS diagnosis to a patient actor during an objective structured clinical examination (OSCE). OSCEs have been shown to be successful in measuring communication skill. (3) Residents completed a self-assessment checklist before and after they watched their own videotaped performance.

Formation of the checklist was influenced by the existing American Academy of Neurology and European Federation of Neurological Societies guidelines (4,5) and the "SPIKES" protocol(6). The checklist comprised the following subgroups: action, communication, empathy. Ethics approval was acquired through the Health Research Ethics Board at the University of Alberta Hospital. Informed consent was obtained from all participating residents.

Results: Twenty-two residents from the Universities of Alberta, Calgary and Saskatchewan participated in the study.

Residents are defined as physicians in training in a five year post-graduate accredited program in neurology. The examiners found that resident performance on the OSCE was often suboptimal, particularly in the areas of communication and empathy. The mean scores for the subgroups were 62% and 65% respectively. The failure rate for the station was also high; approximately one quarter of residents were unsuccessful. There was good correlation between the two examiners' scores for the grand total score, and the subgroup totals for action and communication. Residents' scores (64%) more closely resembled the examiners' after watching their own videotaped performance for grand total of all subgroup gradings.

Discussion: To our knowledge, this is the first study to evaluate use of a videotaped OSCE as a medical education tool in the specific scenario of delivering the difficult diagnosis of ALS. The examiners' gradings of the residents identified areas in which performance in breaking bad news can be improved. The fact that residents' self-assessment was improved after watching their videotaped performance suggests that this may be a helpful addition to neurological training programs.

Conclusion: There is a need for resident education in breaking bad news when communicating the diagnosis of ALS. The exercise of watching their own videotaped performance appeared to be effective in facilitating more accurate self-assessment by the residents.

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P163 WHAT SHOULD WE SAY AND HOW SHOULD WE SAY IT? - EVIDENCE-BASED COMMUNICATION SKILLS IN MND/ALS

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Keywords: communication, evidence, guidelines

Background: ALS/MND patients and families deserve clinicians with good communication skills. Research on what to say and how to say it is sparse. The Practice Parameter for ALS from the American Academy of Neurology (1, 2) has suggested further research in the following areas related to communication:

- patient/caregiver perceptions of how the diagnosis was disclosed
- effects of disclosure technique on future coping
- the impact of cultural and social factors on disclosure technique.

Objectives: In early 2012, the UK's National Institute for Health and Clinical Excellence (NICE) produced evidence-based recommendations on how to optimise patients' experience of healthcare systems (3). As well as reviewing existing research from different parts of the world, including North America, NICE performed a patient experience scoping study which came up with evidence of relevance to optimal communication. The objective of this paper is to see whether the evidence for the NICE guidelines informs the Practice Parameter research questions.

Methods: The NICE recommendations were studied to see if they shed further light on the Practice Parameter research questions relating to communication issues.

Results: Of the 68 guidelines, 58 (85%) were related to some component of communication. This shows the importance of good communication to patient and family experience.

The following themes were of particular relevance to the questions posed by the Practice Parameter and are examined in more detail in this paper:

- respect and empathy from clinicians
- shared non-judgmental decision-making
- negotiating individualised patient and family care according to their particular health and cultural beliefs

Discussion: While ALS is not amenable to cure, aspects of communication are even more crucial to how patients and their families cope with the disease. The remit and design of the NICE review was to produce guidelines which were generic to all disease states and settings. Whilst it is possible that the nature of ALS and its effects on patients mean that generic guidance is not always relevant, the recommendations were specifically formulated to be applicable to any diagnosis carrying a high disease burden.

Conclusion: Evaluating different aspects of communication in ALS through research remains a challenge, especially in a disease which can affect patients' own communication abilities. However, increasing evidence is combined with previous expert opinion in guiding clinicians in this important area. The other on-going challenge, through education, constant updating and practice, is to make such practice widespread.

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P164 THE ROLE OF DESIRED AND ANTICIPATED HOPES IN COPING WITH THE EFFECTS OF ALS/MND

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Keywords: hope, coping, narratives

Background: Hope is regarded as a fundamental element contributing to enhancement of quality of life for people who are dying (1). Studies have explored hope in ALS/MND (2) but limitations of size or design contribute to ambiguity regarding the nature of hope in ALS/MND.

Objectives: To explore the personal experience of living with ALS/MND as documented in personal illness narratives, written by people diagnosed with the illness and examine the phenomenon of hope as it is experienced and displayed.

Methods: We have previously reported the processes used for locating published and unpublished personal illness narratives about life with ALS/MND. The 161 narratives identified were subject to content and thematic analysis. Data management was aided by Nvivo 7 software.

Results: Narrators revealed the presence of two main types of hope, Desired Hope and Anticipated Hope. The first reflects an 'ideal world' situation with often unrealistic expectations, whereas Anticipated Hope involves 'real world' circumstances with a greater possibility of achievement. People with ALS/MND balance these hopes as a means of coping with their illness. There is also evidence of activities and interactions which sustain hope and those which deplete it. Some narrators demonstrated a third type, Pervading Hope, primarily focused on a cure.

Conclusions: This study has demonstrated the importance of balancing different types of hope in coping with the effects of ALS/MND. There are implications for health professionals who must recognise the benefits to patients in having both Desired and Anticipated Hope. Hope should not be dismissed based on a subjective opinion which associates hope in terminal illness with a cure and as such regards hope in ALS/MND as unrealistic. Hope should be regarded as an important resource for coping with ALS/MND.

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P165 USE OF THE ALS SPECIFIC QUALITY OF LIFE INSTRUMENT-REVISED (ALSSQOL-R) TO EVALUATE QUALITY OF LIFE IN INDIVIDUALS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS): A MULTINATIONAL STUDY

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Keywords: quality of life, multinational, normative data

Background: The ALS-Specific Quality of Life Instrument-Revised (ALSSQOL-R) has been validated in English-speaking patients at 12 American ALS clinics. The QOL experience of individuals in other countries has not been explored with this instrument.

Objectives: 1) To determine whether ALSSQOL-R scores differ in patients with ALS in other countries from those in the United States; 2) To examine the relationship of QOL to function in several countries.

Methods: Three Canadian, 1 Swiss and 1 Israeli ALS Center recruited individuals with definite, probable, probable laboratory-supported, or possible ALS. The ALSSQOL-R was administered to subjects in their native language. The range of possible scores was 0 (worst QOL) to 10 (best QOL). Function was assessed using the ALS Functional Rating Scale-Revised (ALSSFRS-R). Pearson correlations and one-way analysis of variance (ANOVA) were calculated. $P < 0.05$ was taken as significant. The study was approved by the Institutional Review Board or Ethics Committee of each institution.

Results: 385 American, 52 Canadian, 34 Swiss, and 19 Israeli subjects were recruited. Mean ages in years were 60.8 (US), 60.9 (Canada), 60.5 (Switzerland), and 58.26 (Israel). Duration of disease in months ranged from 39.9 (US) to 45.8 (Canada), 47.6 (Switzerland), and 34.7 (Israel). There were no significant differences in disease duration or age in the samples. The US mean ALSFRSR score (33.16, SD 7.86) was not significantly different from the Canadian (31.52, SD 8.60) or Swiss (35.47, SD 7.96) samples but was significantly higher than the Israeli sample (26.79, SD 10.83). The mean ALSSQOL-R of the American sample (6.96, SD 1.27) was significantly higher than the Canadian (5.92, SD 1.10), Swiss (5.07, SD 1.0) and Israeli (5.74, SD 0.98) samples ($p < 0.001$).

The American sample demonstrated a positive correlation of QOL and function ($r = 0.191$, $p < 0.001$). Neither the Canadian, Swiss or Israeli samples demonstrated significant correlations between QOL and function.

Discussion and conclusions: Self-reported QOL in patients with ALS as measured by the ALSSQOL-R and the relationships of QOL to function differed in the 4 countries studied. The ALSSQOL-R was developed and normalized using residents of the United States, and it is unknown whether the construct of QOL, the factors which account for it, and the items within those factors are similar among ALS patients from different countries and cultures. Caution is needed when measuring QOL in groups which differ from those engaged to establish normal values for the instrument being used.

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P166 PATIENT ASSESSMENT OF CLINICALLY MEANINGFUL CHANGES: INSTRUMENT DEVELOPMENT

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Keywords: assessment, clinically meaningful changes, instrument development

Background: While analyses of clinical trial data may demonstrate statistically significant differences, it is not known whether such differences are meaningful to study participants or their caregivers. This study was designed to address this question by developing a methodology to evaluate clinically meaningful changes among participants.

Objectives: To identify the extent to which measures of perceived change and their impact on the patient are related to changes on the ALSFRS-R over a 6-month interval.

Method: Patients with definite or probable ALS, who were able to speak on the telephone, and a family caregiver were enrolled at 5 Network sites and were interviewed at baseline, 3 and 6 months. Measures included the ALSFRS-R

with additional ratings addressing each of the 4 subscale domains: perceived amount of change (10-point scale), direction of change (positive or negative), and its impact (10-point scale) as perceived by the patient. In addition, a 4-item scale of Clinical Global Improvement addressing physical, emotional, social and overall life quality was rated (7-point scale). We report selected findings describing change over 6 months.

Results: 60 patients completed the 6-month study. Mean age was 60, 43% were female, 88% completed some college, and 30% were working part or full time. Mean baseline ALSFRS-R was 33 (SD = 7, range = 9–47).

Mean change over six months on the ALSFRS-R was −5.0, and for the 4 subscales as follows: Bulbar = −0.78, Fine Motor = −1.60, Gross Motor = −1.80 and Respiratory = −0.65. Each of the Change and Impact ratings correlated significantly ($p < 0.05$) with the corresponding ALSFRS-R subscale change scores with the exception of the Respiratory domain which showed the least change in ALSFRS-R subscale score. A 1-point decrease on the ALSFRS-R subscales was associated with increased Impact ratings as follows: Bulbar = 2.9, Fine Motor = 3.8, Gross Motor = 4.5 and Respiratory = 1.9. Backwards stepwise regressions were conducted separately for the 4 ALSFRS domains with the Impact rating as the dependent variable. Independent variables included: change in ALSFRS-R subscale, the patient's rating of Change, and 4 CGI scales. For each domain, the patient's Change rating was the only variable that accounted for a significant portion variance (all p values < 0.001), with the exception of Respiratory, where the CGI-physical scale was also significant ($p < 0.05$).

Discussion: These preliminary analyses suggest that patients' ratings of perceived change and the impact of that change, as it pertains to specific areas affected by ALS, may contribute to our understanding of what is clinically meaningful. Ratings that assess broader life areas were not successful in contributing to this understanding. Patients enrolled in this interview study were able to communicate by telephone and thus are not representative of the total ALS population.

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P167 PERCEPTION OF ALS PATIENTS, DOCTORS AND CAREGIVERS REGARDING CLINICAL MANAGEMENT

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Keywords: quality of life, perception, decisions

Background: Noninvasive positive pressure ventilation (NIPPV) increases survival and improves patient's quality of life. Invasive mechanical ventilation (IMV) can prolong survival in ALS. Percutaneous endoscopic gastrostomy (PEG) improves nutrition but also no improvement in QOL is known. The use of these procedures has often been controversial.

Objectives: To evaluate the point of view of ALS patients, carers and physicians about the use of NIPPV, IMV and PEG.

Methods: 30 ALS patients, 30 caregivers and 30 physicians from four different hospitals were examined with a cross-sectional survey. The survey consists of three groups of questions related to the acceptance or refusal the procedures in different levels.

Results: Patients: mean age: 56.5; 76% were males; mean ALSFRS 31.6; duration of disease 568.6 days (sd 354.4); FVC 72.6. 90% of ALS patients have a carer. All doctors agree to use NIPPV in all patients and situations and 96% of them to use the PEG for nutrition. With regard to NIPPV, 50% of patients agree with the opinion of physicians and caregivers and 26.6% agree regarding PEG. IMV was the most controversial procedure, only in 20% of patients accepted IMV like the caregivers and doctors. Physicians showed very different opinions: from acceptance to the rejection of this procedure.

Conclusion: The perception of the patients, caregivers and doctors in relation to PEG, IMV and NIPPV is very different. Decisions should be taken by all together.

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P168 FAMILY CARER PERSPECTIVES OF HOSPITAL CARE FOLLOWING A DIAGNOSIS OF MND: A SECONDARY ANALYSIS

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Background: People with MND can experience a number of hospital admissions throughout the course of their illness. These may be planned admissions for specific interventions or emergency hospitalisation as a result of the development of concomitant illness or advancement of their condition.

Objectives: The aim of this study was to explore experiences of hospitalisation following a diagnosis of MND from the perspective of family carers of people diagnosed with the illness.

Methods: The study reports on a secondary analysis of pre-existing data from two previously published qualitative studies conducted separately by the authors in Northwest England. During discussions between the primary researchers, the extent to which experience of hospitalisation was present in the data became apparent. Significant similarities were evident in the datasets which made it viable to pool data and undertake a new and distinctive analysis; this topic was not the primary focus of the previous studies. Only interview transcripts that featured experiences of hospitalisation were included in the secondary analysis; the pooled sample therefore consisted of 10/11 bereaved carers from study 1 and 10/10 bereaved carers together with 3/18 current carers from study 2. The latter study also involved interviews with people with MND, but these data were not included in the secondary analysis as the focus was on carer perspectives.

Results: A number of issues emerged from the analysis. There were distinct differences in standards of care between planned and unplanned admissions and within specialist and general wards. Basic care was lacking at times as was the understanding of how to care for someone with MND. Relatives became educators to overcome deficits in professionals' knowledge. Communication between staff and some patients/relatives was unsatisfactor

preferences disregarded at times. The final memories of loved ones were affected by relatives' experiences of hospital care at that time.

Conclusions: This study has, for the first time, provided insight into the perspectives of carers of people with MND specifically regarding hospital care received subsequent to a diagnosis of MND. There are distinct implications for the delivery of hospital based care, whether it is planned or unplanned.

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P169 ILLNESS BURDEN IN PATIENTS WITH ALS AND THEIR CAREGIVERS: A WEB-BASED SURVEY

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Keywords: quality of life, caregiver burden, patients with ALS

Background: There are compounds in development for the treatment of amyotrophic lateral sclerosis (ALS). In addition to proving a beneficial effect on survival and function, research is needed to quantify economic losses, caregiver burden, and quality of life impact associated with ALS at different stages of the disease. We sought to investigate lost productivity, caregiver burden, and quality of life among patients with ALS (PALS) and caregivers (CALS). This survey is designed to explore relationships between PALS disease severity, functional impairment milestones and the socioeconomic burden on CALS.

Methods: PALS and CALS who participate in PatientsLikeMe, an online data-sharing platform for people with serious illnesses, were invited to take an online survey. Inclusion criteria for participation in the survey were: US residence, activity on PatientsLikeMe in the past 120 days, a self-reported diagnosis of ALS or identification as a CALS who serves as the main provider of help and care to a person with ALS; CALS may be a spouse, parent, child, other relative, friend, or professional caregiver. Both PALS and CALS completed information on demographics, the Health and Work Performance questionnaire (HPQ), and the EuroQoL EQ5D-3L. Additionally, PALS completed the ALSFRS-EX (Extension items) and ALS Assessment Questionnaire (ALSAQ-5). CALS completed the Caregiver Burden Inventory (CBI).

Results: Recruitment is ongoing throughout 2012 but an interim analysis was performed with 31 matched pairs. Both groups were similar in age (mean age, 57 years) with CALS slightly more likely to be female. Mean ALSFRS-R score in PALS was 25.6, although the range of scores was wide. As expected, PALS reported a worse quality of life on the EQ5D-3L VAS than their caregivers (61.3 vs 77.9; $P = 0.002$), though results showed a great deal of variability. More CALS were working for pay than PALS (53% vs 16%; $P < 0.001$). PALS primarily contributed via housework; CALS shouldered a variety of responsibilities in addition to caregiving, including employment, childcare, and housework. Most CALS (87%) reported having had to change work in some way owing to their PALS' illness, with many using vacation days (54%), reduced working hours (46%), or resigning from their job (33%). In terms of caregiver burden, there was a relationship between the negative aspects of caregiving (e.g. burden) and ALSFRS-R (R-squared = 0.338, $P < 0.001$); 52% of CALS were above the threshold for the CBI's cut off of 36 for 'caregiver burnout' warning.

Discussion and conclusions: Preliminary results suggest a high level of burden for CALS across a number of domains (e.g., socioeconomic impairment, personal responsibilities, anxiety, depression, and pain). This project aims to describe the impact that ALS can have on PALS, CALS, and society more broadly, with a secondary goal of identifying specific inflection points in the disease that might trigger significant PALS and CALS burden.

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P170 LONGITUDINAL ASSESSMENT OF CAREGIVERS OF PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: caregiving, quality of life, assessments

Background: As amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS) progress, caregivers provide increasing physical and emotional supports to patients. The impact over time on caregivers is not clear.

Objectives: To understand the evolution during the ALS/PLS disease trajectory of caregivers' perceptions of their ability to assist patients, and on the caregivers' supports, health, and quality of life (QOL).

Methods: This was a prospective, IRB-approved study in which a questionnaire was sent to caregivers of patients with ALS or PLS prior to each outpatient clinic appointment, usually once every 3 months. Caregivers were asked to assess their level of concern (worry, anxiety, unease) with providing each of 17 caregiving activities. Caregivers also were asked to identify those activities which were supportive to them, and to answer questions about their health and quality of life (QOL).

Results: 239 caregivers were enrolled. The mean age was 58.8 years, 67% were women, and 78.6% were spouses. Factor analysis resulted in two scales which accounted for 43.19% and 13.01% percent of the variance in level of concern with providing caregiving activities. Scale 1 included 9 activities of daily living (ADL) such as assistance with medications, feeding, and mobility; chronbach's alpha = 0.91. Scale 2 included 8 psychosocial activities such as emotional/spiritual support, encouraging the patient to participate in activities, and assisting with insurance issues and community programs; chronbach's alpha = 0.87. Three assessments over 6–8 months were completed by109 caregivers for longitudinal analysis. The mean age was 58.8 years, 66% were women and 78.9% were spouses. Repeated measures analyses of variance found significant increases in concerns over time for both the ADL and Psychosocial Task subscales ($p < 0.004$), with significant effects for the time by gender interaction for both scales ($p < 0.02$), in which the concerns of the women increased and approached the higher levels of the men. There was a significant ($F(1,38) = 5.55$, $p < 0.02$) gender effect for the number of supportive activities engaged in, with women greater than men at each point, although this diminished over time as women gave up some of these activities. There were no significant changes over time in QOL and health measures, but women had somewhat higher

QOL scores at all points, and women's reports of depression began to increase relative to men's at the third time point.

Discussion and conclusions: Caregivers became more concerned about their ability to provide caregiving activities over time, with a decrease of the initial advantage of women over men. Women engaged in more support activities than did men, but this decreased over time. Caregiving in motor neuron disease appears to be a dynamic process. Ongoing monitoring and re-assessment of caregivers is necessary to identify appropriate interventions throughout the disease trajectory.

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P171 PATIENTS' AND CAREGIVERS EXPERIENCE OF MECHANICAL IN-EXSUFFLATION

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Keywords: qualitative research, cough, respiratory therapy

Background: The progression of muscle weakness in amyotrophic lateral sclerosis (ALS) leads to respiratory complications with airway secretion encumberment from ineffective coughing. Mechanical in-exsufflation (MI-E) can be applied to help eliminate secretion for patients with impaired cough. The introduction of technology from the hospital setting into the home leads to increasingly specialized and complex challenges for patients, informal caregivers and community healthcare professionals. How does the use of medical technology influence on the interaction between different actors in the home arena? And how does technology influence patients' experiences of their illness? The complexity of managing a severe disease, such as ALS, will challenge healthcare professionals in offering an optimal treatment for these patients.

Objective: To examine patients with ALS and their caregivers' experiences when using the MI-E to enhance coughing. The study focused on user confidence, and what influence the MI-E had on relationships between the actors, as well as on the patients´ illness perception.

Methods: We did a qualitative study, using semi-structured in-depth interviews with patients with ALS and their caregivers. Eleven interviews were performed with five patients and six caregivers. The material was analyzed using systematic text condensation. Actor-network theory (ANT) was used as a framework to understand the concept of interactions in heterogeneous network when technology is used in medical treatment.

Results: We organized the findings into three themes; 1) Trust and confidence, 2) competency and 3) usage patterns and effect. The findings showed that confidence and user competency were essential aspects for both patients and caregivers in order to be comfortable using the MI-E. The relationships between patient and caregiver affected the experience of using the MI-E. Both patients and caregivers reported that the MI-E had positive and negative impact on their daily life. It was a challenge to achieve good transference of skills in operating the MI-E to every caregiver when there were many caregivers involved. The use of the MI-E differed due to various reasons beyond the effect on eliminating secretion. Using the MI-E, at times, made the disease progression more apparent for the patient.

Discussion and conclusions: These findings show that the complexity of caring increases when a technological device is introduced into the patient's home. By applying a sociological perspective, the diversity in the interaction between humans and technology, beyond the medical intent of the technical device, can be displayed. Healthcare professionals at all levels need to take into account social and individual aspects when introducing the MI-E to patients with ALS. Further research is needed to improve the knowledge of how interactions in a network impact on patients and caregivers' perception of purpose and confidence when medical technology is introduced.

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P172 PHYSIOTHERAPY AND EXERCISE TO PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Physiotherapists in Denmark have an important role in guidance regarding respiration, exercise and assistive devices to patients with amyotrophic lateral sclerosis (ALS). There is no current consensus among physiotherapists in Denmark about which treatments to use and when to use them. In the literature very few papers describe treatment given by physiotherapists to patients with ALS.

The aim of this study is to investigate which treatments are used, the evidence for these treatments and whether it is possible to create consensus on a clinical guideline in Denmark built on clinical experience and evidence.

The study started on 1 January 2012 with expected completion in December 2012.

Objectives: 13 hospitals in Denmark diagnose and treat patients with ALS. They all have an ALS-team with at least one physiotherapist. All 13 teams are included in this study. The populations in the area of the 13 hospitals are comparable.

Methods: The study consists of three steps:

Step one: 16 team-physiotherapists have received and answered a semi-structured questionnaire about their treatment strategies for patients with ALS.
Step two: A systematic search for literature in the databases Medline, Cinahl, Cochrane and Pedro was made. Publications of evidence level Ia and Ib were read systematically and critically.
Step three: All team-physiotherapists were invited to a workshop with focus on discussion of evidence and treatment strategies.

Results: The aim of this study is to reach consensus about the physiotherapy treatments for patients with ALS in the hospitals in Denmark and to publish a national clinical guideline.

Step one: 16 out of 19 physiotherapists answered the questionnaire. All physiotherapists give instructions to patients in exercises and respiratory aid and support, but the instructions were different in each of the 13 hospitals.

Step two: 7 papers about exercises and 14 papers about respiratory support and exercise were included for critical reading. There was good evidence for non-invasive ventilation (NIV) but no guidelines for exercises were found.
Step three: 12 hospitals were represented by physiotherapists at the workshop. Agreement was reached on a clinical guideline.

Knowledge from clinical practice and evidence from the literature will result in a national clinical guideline. The guideline will include optimal time for respiratory aid and support, guidance on respiratory support, training instructions and cough-support.

Discussion and conclusion: We have found good clinical practice points for training in our literature study but no strong evidence. Several studies recommend NIV as a respiratory support also in the early stage of ALS. In the future, physiotherapists in Denmark will focus on earlier use of NIV, regular tests of lung function and recommendations on exercise.

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P173 AQUATIC PHYSICAL THERAPY PROGRAM FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Keywords: aquatic physical therapy, function, pain

Background: Despite a recognized need for a physical therapy (PT) program to care for people with motor neuron disease (MND), access to PT is often delayed. The aim of aquatic PT in patients with ALS is to maintain the patients' health related to functionality, and quality of life (QoL) as well as minimizing their fatigue.

Objectives: To verify the beneficial effects of an aquatic PT program for ALS patients as applies to increased strength and function; as well as minimizing pain and fatigue, and overall QoL improvements.

Method: A prospective study of patients with sporadic ALS (n = 4) was conducted. Patients were submitted to a 12 week program consisting of 24 sessions of aquatic PT in a heated 33°C pool, twice a week, for 45 minutes each session. Standardized assessments were used to evaluate patients before and after the 12 week treatment period by the following scales: ALSFRS-R, Manual Measurement Test for strength, Fatigue Severity Scale and Visual Analogic scale for pain.

Results: Evaluation of the patient questionnaires revealed that muscle groups were able to maintain their strength, as well as most ALSFRS-R items (33.5 ± 2.5 before and 30.25 ± 5.1 after), and QoL. All patients stated that they had decreases in both pain and fatigue.

Conclusion: Recognizing ALS as a progressive and incurable disease, a program of aquatic PT designed specifically for this group of patients produced exemplary results. Aquatic PT both delayed the evolution of the disease and helped maintain muscle strength, physical function and QoL as well as decreased pain and fatigue.

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P174 A SURVEY OF HEALTH CARE PROFESSIONALS' VIEWS ON EXERCISE AS A TREATMENT FOR PERSONS DIAGNOSED WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: survey, exercise, therapy

Background: Few published studies exist that examine the effects of exercise on persons with amyotrophic lateral sclerosis (ALS). Available studies have not shown adverse effects of exercise, but they also have not shown conclusive long-term benefits. (1,2) As there is no standard of care, health care providers must decide on their own whether exercise should be prescribed to this population.

Objectives: The purpose of this study is to clarify what exercises, if any, health care professionals in the United States (US) are prescribing to persons diagnosed with ALS and determine what research and experience-based views professionals working regularly with persons diagnosed with ALS hold.

Methods: A survey of 12 questions regarding ALS and exercise and 3 questions requesting demographic information was developed. A link to this survey was sent to ALS Association Chapters and Muscular Dystrophy Association ALS Clinics in the continental US. A letter sent with the link asked all physical therapists, occupational therapists, neurologists and physiatrists associated with the center to fill out the survey. Survey Monkey collected data.

Results: After incomplete responses were omitted, 109 responses were used to compute results. Thirty-nine percent of respondents said they only recommend active exercise to persons with ALS if exercise was part of the person's lifestyle pre-diagnosis. Thirty-six percent of respondents said they recommend active exercise to all persons with ALS, but 92% clarified this statement with comments such as "depending on fatigue level". The other 24.7% of respondents do not recommend active exercise to any persons diagnosed with ALS. Only 20% of respondents think exercise maintains strength while less than 5% think exercise improves strength in persons with ALS. Contrary to prior published research examining exercise and ALS, many respondents reported that, in their experience, exercise by persons with ALS could have adverse effects, such as falls, fatigue and rapid strength loss. Only 32% of respondents listed previously published literature when asked about articles they refer to regarding ALS and exercise.

Discussion: There is some variation in exercise prescription among healthcare professionals who have experience with persons diagnosed with ALS. Many professionals believe that exercise prescription should be done on a case-by-case basis for persons with ALS as both positive and

negative effects can result. Experience has not shown conclusively that exercise is beneficial for maintaining or increasing strength in persons with ALS.

Conclusion: Further research is needed to standardize prescription of exercise in persons with ALS. Development of an algorithm, taking variables such as lifestyle and fatigue into consideration, rather than a general prescription may be most effective.

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P175 CLINICAL FEATURES OF FATIGUE IN PATIENTS WITH MND

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Keywords: fatigue, sleepiness, psychological distress

Background: Fatigue is a persistent and disabling symptom for MND patients. Research studies evaluating the relationship of fatigue and other disease factors, including depression and functional status, have provided conflicting information.

Objective: To clarify the relationship between fatigue and other features of MND in a large cross-sectional study using a disease-specific measurement tool for fatigue in MND.

Method : A pack containing the Neurological Fatigue Index for Motor Neurone Disease (NFI-MND) summary scale, the Hospital Anxiety and Depression Scale (HADS), the Amyotrophic Lateral Sclerosis Functional Rating Scale – revised (ALSFRS-R), the Epworth Sleepiness Scale (ESS) and questions regarding sleep, disease presentation and other demographic factors, was mailed to patients at five neurology care centres in the UK.

Results: Data from 298 respondents (54.7% response) were analysed. Fatigue was positively correlated with daytime sleep ($r = 0.13$, $p < 0.0001$) and sleepiness ($r = 0.40$, $p < 0.0001$). There was a strong relationship between fatigue and psychological distress (anxiety and depression) ($r = 0.57$, $p < 0.0001$). Fatigue was not correlated with age or disease duration. Levels of fatigue were significantly higher if patients experienced respiratory symptoms ($t = -3.49$ $p = 0.001$). Patients with limb symptoms experienced higher levels of fatigue than those with bulbar presentation only ($t = -6.29$, $p < 0.0001$). Fatigue correlated mildly with functional status ($r = -0.18$, $p = 0.031$). Patients that slept for 30 minutes during the day and seven hours at night reported the lowest levels of fatigue.

Conclusion: Relationships were apparent between fatigue, daytime sleep, sleepiness, psychological distress and functional status. Knowledge of these relationships may be used to inform further work to develop interventions for patients suffering from fatigue.

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P176 PAIN IN MOTOR NEURON DISEASE (MND) – A PILOT STUDY

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Keywords: symptom, pain, Brief Pain Index – Short Form

Background: Pain can occur in almost 70% of patients with MND and is often a frequent symptom in the later stages of the disease. Although effective clinical management of MND requires different aspects of pain to be identified and possibly treated, the character of the pain is insufficiently studied.

Objectives: The aim of this pilot study was to describe the character of pain in MND in a limited Swedish population, specifically the location and severity of pain, the interference in daily life, and possible sex differences regarding the experience of pain.

Methods: The study was conducted at the Department of Neurology, MND-unit, Uppsala University Hospital, Uppsala, Sweden. Data were collected from personal files through the Brief Pain Inventory- Short Form. Thirty-six participants (mean age 61.5 years, 56 % men) fulfilled the requirements: the average time from diagnose was 2 years.

Results: 72% of the patients experienced pain, primarily in the legs (23%) and shoulders (23%). Pain intensity ranged from Md = 0 (minimum pain) to Md = 7 (worst pain), with an average pain sensation of Md = 3. For a variety of daily activities, pain had the greatest influence on"general activities, the ability to walk and sleep". There was no significant difference in the average estimate of pain between men and women ($p = 0.402$).

Conclusion: A majority of the patients experienced pain, despite an early stage of the disease. This highlighted the need for including clinical assessment methods for adequately determining the character and consequence of pain.

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P177 A CLASSIFICATION SYSTEM TO ASSESS THE DEGREE OF DISABILITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: functional scale, disability, factor analysis

Background: Establishing the degree of disability in patients with amyotrophic lateral sclerosis (ALS) is useful for defining treatment plans and rehabilitation guidelines in these patients.

Objectives: To propose a disability classification system for patients with ALS based on the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R).

Methods: A sample of consecutive patients with possible, probable and definite case definition of ALS according to clinical and electrophysiologic criteria was assessed. In every patient, age, gender, initial topographic presentation, time from onset of symptoms to the moment of assessment, level of clinical certainty and total score of the ALSFRS-R Spanish version were determined. A principal component factor analysis of the ALSFRS-R was carried out to establish the different domains of the scale and to choose its most useful variables. With the chosen variables, groups of disability were defined. Correlation between these groups of disability and the ALSFRS-R total score, rate of progression of the disease (DFS) and muscle strength median (according to the Medical Research Council) was assessed.

Results: Eighty-one patients (male 61%) were assessed. Initial topographic presentation was 16 (20%), 35 (43%) and 30 (37%) for bulbar, cervical and lumbar segments, respectively. Clinical certainty was 15 (19%) possible, 22 (27%) probable and 44 (54%) definite. Factor analysis of the ALSFRS-R showed three domains that accounted for 72% of variance: 1) activities related to bulbar functions, 2) activities related with upper limb dexterity and 3) activities related to gross motor function. One activity was selected from each of these three domains according to its weight within the domain. Swallowing, cutting food and handling utensils and walking were selected from domains one, two and three, respectively. These three activities were dichotomized as dependent (ALSFRS-R score of 0, 1 and 2) or independent (ALSFRS-R score of 3 and 4). Afterwards, four groups of disability were defined: dependent in the three activities (12 patients, 15%), independent in one activity (9 patients, 11%), independent in two activities (28 patients, 35%) and independent in three activities (32 patients, 39%). These four groups of disability were statistically correlated to the level of clinical certainty ($p = 0.003$, $r = 0.32$), rate of progression of the disease ($p = 0.003$, $r = 0.32$) and muscle strength ($p = 0.000$, $r = 0.5$).

Conclusion: This four-group classification system of disability based on three selected activities from the ALSFRS-R discriminates functionality of patients with ALS, is easy to use in everyday practice and has an association with the level of clinical certainty, DFS and muscular strength.

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P178 EXPLORING THE APPLICATION OF SENSECAM TO INDIVIDUALS WITH MND

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Keywords: occupational therapy, adaptations, technology

Introduction: Motor neuron disease is a disease which rapidly erodes people's ability to participate in everyday occupations. It is the occupational therapists (OT) role as a part of the multidisciplinary team management of the MND patient, to assess and explore to optimum management of the patient's difficulties in everyday life. The specialist OT at the Barts and the London MND Centre is able to provide assessment and intervention recommendations but this does not allow for full, timely and appropriate assessment of an individual's occupational environment in order to support their continued engagement in important life roles. Opportunities exist to consider how innovative technology may be of best use in providing health services to individuals living with MND. SenseCam is a small, wearable digital camera which captures an electronic record of a wearer's day. The device takes photographic images automatically, without user intervention, whilst it is worn on a lanyard around the user's neck.

Objectives: The aim of this project was to evaluate the occupational therapy service provided for individuals living with MND to ensure that it met service-user needs. As part of this intervention, the SenseCam was utilised as an alternative means of obtaining detailed information about a person's environment/s and how they perform a range of functional activities.

Methods: The project involved the appointment of a full-time occupational therapist for a 12-month project to provide occupational therapy services in the Barts and the London MND Centre. Service-users were given the opportunity to use the SenseCam as a component of their occupational therapy intervention. Evaluation was conducted across a range of methods including clinical effectiveness, activity analysis of the SenseCam images and service-user feedback.

Results: Service-users utilised the camera for varying amounts of time and in varying environments. Activity analysis of the digital images revealed beneficial use of the SenseCam in relation to the following areas of occupational therapy assessment and intervention: postural management; environmental assessment; risk identification and management; fatigue management and ergonomics; carer and family education; and management of disease progression.

Discussion: Preliminary analysis of service-user feedback has supported the extension of the occupational therapy service, which has been enabled by the use of the SenseCam as a therapeutic adjunct. Additionally, the SenseCam images have been useful in informing timely and appropriate involvement of the multi-disciplinary team, and supporting early recognition and management of functional difficulties.

Conclusion: The SenseCam utilisation has been demonstrated to have notable potential in supporting comprehensive occupational therapy assessment and intervention for individuals with MND.

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P179 A VIRTUAL TELEVISION REMOTE VIA COMPUTER FOR INDIVIDUALS WITH ALS/MND

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Keywords: environmental control, assistive technology, television access

Background: For many individuals living with amyotrophic lateral sclerosis (ALS), maintaining independence is an important goal. This includes independence as it relates to controlling devices in the environment. One activity that can become quite difficult is the use of a standard remote control f

due to the strength and dexterity required to press buttons. While there are switch adapted controlling systems available, these systems are restricted by expense and limitations in their functionality. We have set out to design a flexible, low-cost controller that can be plugged into any Microsoft computer. We are using television control as proof of concept.

Objectives: The objective is to design a low-cost virtual remote device (NIXBOX) capable of television control and expandable to other infrared (IR) remotes to enhance the independence of individuals living with ALS/MND.

Materials and methods: This system is designed for the user to control their television with a switch via a virtual remote on the computer screen. It utilizes two types of software and hardware. The software used is Arduino and LabVIEW. Arduino receives, processes and sends IR signals. LabVIEW controls the graphical user interface (GUI), signal storage and user customization. The two types of hardware are an Arduino microcontroller and electrical components (3.5mm audio jack, 38kHz IR detector, 940nm High-Output IR LED, Green LED, 10kOhm Resistor, two 330Ohm Resistors). There are three steps necessary to complete the task of controlling the television. In the first step, the virtual remote learns the remote control's IR signals for each button. Next, the virtual remote stores the signal data onto the computer. The final step allows the GUI to control the television through the computer by transmission of IR signals. The basic television remote is displayed as a virtual remote on the monitor. It is designed in row-column form for scanning compatibility using any type of switch. Each button on the virtual remote is linked to a unique signal that is sent to the television. This device connects to the computer via USB. Combining the hardware and software technologies, environmental control is possible.

Results: We have built a prototype NIXBOX that is being demonstrated and tested for user feasibility.

Discussion and conclusions: We have designed an inexpensive, versatile, computer-based IR controller that has been used as a television remote for proof of concept. The system is designed to work with any IR remote and has capabilities of further implementation for other environment controls. This device is easy to set-up and it can accommodate to changing levels of function. This system will be accessible to people living with ALS/MND for increasing their independence and at a relatively low cost.

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P180 MULTIDISCIPLINARY QUALITY CARE AFTER THE POWER WHEELCHAIR DELIVERY: CHANGES REQUIRED OVER TIME

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Keywords: wheelchair, rehabilitation, durable medical equipment

Background: Rapid progression of ALS/MND means the power wheelchair must be flexible for long-term needs for comfort and function. No current articles exist on what types of changes are required and how often the changes must occur after a client receives a power wheelchair.

Objectives: To report the types and frequency of changes required after receiving power wheelchairs and to develop guidelines for what is required for long term effective use, comfort and function of their power wheelchairs as the disease progresses.

Methods: Chart review was completed to compile records of power wheelchair repairs and changes in type and frequency. All power wheelchairs were ordered originally with experienced therapy evaluators and vendors to have the maximum long-term flexibility.

Results: From 2010 to present day, 92 ALS patients received power wheelchairs out of 145 seen through the multidisciplinary clinic. The average patient with a wheelchair received 5.75 (SD 1.3) changes to their wheelchair in this 2-year time period, and 4.0 (SD 2.4) repairs in the same period. Some of the changes made include a pressure relieving cushion, headrest lateral or forward supports, more supportive armrests, thigh guides, and alternative drive controls including switches. Clients were re-evaluated for necessary changes at every clinic visit by experienced therapy and vendor staff. Repairs were generally minor, and completed at the clinic visit as well.

Discussion: Most insurances in the US will pay for medically necessary changes to be made to the chair once it is purchased. Patients require skilled assistance to help them make these changes to maintain full control over the chair as well as comfort and function over time, and this requires not only knowledgeable clinic staff, but also an experienced vendor.

Conclusion: We feel our multidisciplinary ALS/MND clinic has a responsibility to help provide evidence for the long-term needs of ALS/MND patients and their power wheelchairs because of the full time experienced therapy staff, as well as competent dedicated vendors. Our patients desire to use and control their wheelchairs fully, with whatever means they may.

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P181 UPPER EXTREMITY ORTHOSES IN ALS/MND

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Keywords: orthosis, splint, function

Background: Occupational Therapists often provide custom and pre fabricated Orthoses for support and bracing of weak and ineffective joints or muscles of the upper extremity in the management of ALS/MND. The purpose of the orthosis is to decrease the effects of muscle imbalance to provide greater ease in performance of activities of daily living (ADLs), prevent joint contracture, and to relieve pain.

Objectives: The objective of this study is to perform a systematic review of the literature as well as to draw on experiences in the ALS clinic to determine which upper extremity orthotic devices are useful for pts with ALS/MND.

Methods: A systematic review of the literature available on Medline, EMBASE, Google Scholar, PubMed, and Cinahl was performed; 32 articles were appraised by one author according to a standard format. Observational, qualitative, and quantitative studies were included. In addition, the author

drew on her own experience over the past 25 years with ALS/MND patients.

Results: No randomized controlled trials or controlled clinical trials were identified. A summary of descriptive and qualitative studies that relate to upper extremity orthoses as well as results in the author's experience will be discussed.

Discussion and conclusions: Although there were no randomized controlled trials, five upper extremity orthoses are discussed. These will be described with photographs and case descriptions in this poster. The orthoses can be categorized as volar and dorsal wrist, proximal interphalangeal (PIP) finger extension, night resting, thumb opposition, and orthoses designed for a specific function. Further research is needed into appropriate study designs for the use of upper extremity orthoses and the best manner of assessing outcomes with this intervention. Case experience (Level 5 Evidence: "Expert Opinion") reveals functional advantage to use of the above stated orthoses.

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P182 FALLS IN COMMUNITY DWELLING AMBULATORY INDIVIDUALS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND PRIMARY LATERAL SCLEROSIS (PLS)

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Keywords: falls, balance, injury

Background: Falls in ALS and PLS have not been well studied, but may lead to a reduction in activities, increased dependence on others, or more severe health consequences.

Objectives: To determine the prevalence, characteristics, and consequences of falls in a sample of people with ALS or PLS and compare characteristics of fallers and non-fallers.

Methods: We conducted a prospective study of ALS and PLS outpatient's attending our ALS clinic. Eligible subjects were able to stand without assistance, and had an ALSFRS-R walking score of two or greater. All maintained a daily falls calendar and reported falls by telephone. In-person assessments were performed every 3 months with the Berg Balance Scale (BBS), Timed Up and Go (TUG), Activities Specific Balance Confidence (ABC) Scale, ALSFRS-R (total score, and fine and gross motor sub-scores), quality of life, manual muscle testing, and the upper motor neuron score. Pearson correlations ($p < 0.05$) and stepwise regression were employed for analysis. The Penn State Hershey Medical Center Institutional Review Board approved the study.

Results: Fifty seven patients were enrolled - 52 ALS and 5 PLS (65% men). Mean duration of disease at first study evaluation was 61 months for PLS and 29 months for ALS. Over 14 months, 136 falls were reported in 33 subjects (66%). While a greater percentage of women than men fell (60 vs. 56%), overall men had more falls (71%) than the women (29%) with 16 of the 18 subjects that had 3 or more falls being male. 62 of the falls (45%) resulted in injuries, seven of which were severe (fractures), 13 moderate (laceration or sprain), and 42 minor (bruise or abrasion not requiring medical attention or alteration in function). 28 participants (49%) reported a past history of falls at study entry, while 12 participants experienced their first reported fall during the study. Thus, a prior history of falls is indicative of increased risk for a second or repeated fall. A significant correlation was found between falling and the ABC, ALSFRS-R total, and ALSFRS-R gross motor sub-scores ($p < 0.05$). By stepwise regression, the ALSFRS-R gross motor sub-score ($R^2 = 0.24$, $p = 0.01$) was most predictive of future falls. Follow-up regression analysis found that the change in ALSFRS-R gross motor score between the baseline and second visits was a significant ($R^2 = 0.20$, $p < 0.05$) predictor of increased risk for falling before the next clinic visit.

Discussion and conclusions: Patients with ALS and PLS are likely to experience one or more falls, injuring themselves nearly half of the time. A decline in gross motor sub-score on the ALSFRS-R, and self-confidence using the ABC may provide clinicians opportunities for timely falls prevention intervention.

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P183 ADAPTATION AND SERVICEABILITY OF THE MAXIMUM INSUFFLATION CAPACITY (MIC) TRAINING TO THE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: maximum insufflation capacity training, respiratory physiotherapy, peak cough flows.

Background: Maximum insufflations capacity (MIC) is the maximum volume of air stacked in the patients' lungs beyond spontaneous vital capacity. It has been suggested that MIC training is useful for patients with muscular dystrophy in maintaining the elasticity of lung and chest-wall range of motion.

Objectives: To investigate adaptation and serviceability of the MIC training for patients with ALS.

Methods: The method of MIC involved patients inhaling 3 to 5 times continuously through the Bag Valve Mask (BVM) until their mean airway pressure reached 40 to 50 cm H_2O, then, following air stacking, to exhale in one breath. Subjective difficulties of performing MIC was assessed using a Visual Analogue Scale (VAS). Speech and swallowing scores were assessed using the ALSFRS-R (3–4 scores; non-bulbar, 0–2 scores; bulbar) and Video fluorgraphic examination (VF) in order to examine MIC training serviceability. The patients who were thought to have adapted to MIC training had further examinations of Volume Capacity (VC) and Peak Cough Flows (PCFs) 5 times each before, during and after their operations. At the end, we present two case studies of patients we succeeded in assessing long term.

Results: Eight patients were included in the multidisciplinary study. As an entry criterion, they were diagnosed clinically probable ALS (6 patients) and clinically possible ALS (2 patients), according to the revised El Escorial, and

participated in physiotherapy sessions with prospective study during April 2010 to May 2011.Their mean age (y.o), ALSFRS-R, number of lapsed years from onset (years), % predicted vital capacity (%) were 55 ± 3.4, 32.1 ± 7.1 (44–22), 2.1 ± 2.8 (0.9–3.8), 50.6 ± 18.1 (29.1–80.1), respectively.

We found MIC training was suitable for non-bulbar type ALS, with over 5 VAS, and over 3 on the ALSFRS-R for speech and swallowing, and no sign of miss-swallowing at VF.

MIC training also showed a significant increase in VC and PCFs, not during training alone, but also in its post-operation results compared with its pre-operation results. On during operation, the results of VC and PCFs reached 172.8 ± 41.1% and 189.2 ± 84.4% respectively, and on post-operation the results of VC and PCFs reached 118.2 ± 23.1% and 110.8% respectively in comparison to being as 100% on pre-operation results of VC and PCFs. Moreover, the two patients who performed MIC training over a longer term showed excellent potential exists to not only maintain and improve MIC but also keep up and increase PCFs even though their VC had been dropping from 74% to 48% for year, and 74% to 61% for 6 months respectively.

Discussion and conclusions: This study showed MIC training has a benefical effect on non-bulbar ALS patients to keep up their chest compliance and mobility as one aspect of their respiratory physiotherapy.

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P184 USABILITY OF BIOSIGNAL COMMUNICATION DEVICES FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) USING TRACHEOSTOMY VENTILATION

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Keywords: biosignal-based communication devices, brain-machine interface, communication stage

Background: Research on ALS patients using communication methods through brain-machine interfaces (BMI) and other systems using biosignals has been limited. Few studies have examined biosignal devices in the severely disabled.

Objective: The purpose of this study was to examine the usability of biosignal-based communication devices for ALS patients, focusing on the relationship between responses of patients and appropriate levels of output signals.

Methods: Subjects were six ALS patients (four males and two females) undergoing tracheostomy positive pressure ventilation (TPPV). Their mean duration of the disorder or post-diagnosis survival = 11.3 years, and a mean duration of TPPV use = 8.0 years. Study details: Responses by the patients, their ability to communicate, and results of the trial use of the following devices: 1) biosignal-based MCTOS (a bio-switch system produced by Technos in Japan); 2) Kokorogatari (equipment designed to express "yes" or "no" using cerebral blood flow in the brain, when thinking deeply; developed by Hitachi in Japan), and 3) Neurocommunicator (an EEG-based BMI system developed by AIST). The subjects were classified into five stages (Stages I to V) according to the level of communication ability. The researchers evaluated use of the three types of communication devices.

Results: Three patients at "Stage I" were able to communicate using sentences. They could convey thoughts and feelings by means of a word board and other communication devices. Stage III included one patient with the ability to express "yes" or "no" by eye movement. One patient was a "Stage IV" and was sometimes able to express "yes" or "no" through eye movement. Stage V consisted of one patient who was unable to communicate due to total paralysis. Three patients at Stage I were able to use all equipment. The Stage I patients indicated that the Kokorogatari, "cannot express anything, but 'yes' or 'no,' and is too slow. "Accuracy of Kokorogatari for the patients on stages IV and V was 70 to 100% at the chance level of 50%. Accuracy of Neurocommunicator for those on the stage I and III was 50 to 100% at the chance level of 12.5%.The accuracy of these systems might be dependent on the noise interference and/or the consciousness level of the patients.

Discussion and conclusion: Stage IV and V groups were able to respond using the Kokorogatari. This suggested that biosignal-based communication methods served as a means of conveying thoughts and feelings by totally paralyzed patients using TPPV. Further studies should examine the time required for patients to adapt to equipment and changes in their responses over time, since the appropriate timing for introducing devices and frequency of their use vary depending on the patient. We recommend early initiation of the devices in tracheostomy patients and to follow the patients' progress.

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P185 OPINIONS AND BEHAVIORS OF JAPANESE AND AMERICAN ALS CAREGIVERS REGARDING TRACHEOSTOMY WITH INVASIVE VENTILATION (TIV)

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Keywords: tracheostomy, patient decision-making

Background: International studies have found widely disparate rates of TIV between countries, between areas within countries, and even in the same hospital. When TIV is initiated, it is often done emergently without advance directives. Many contributory factors have been suggested, including contextual, patient and family influences.

Methods: We conducted national surveys of American and Japanese patients and caregivers, selected because American patients have among the lowest, and Japanese the highest rates of TIV. Here we present selected findings from the caregiver survey. Two groups of patients were included: recently diagnosed, and patients with respiratory problems. During routine

clinic visits, patients accompanied by a family member were asked whether they knew about TIV, and if so, were invited to participate. Five geographically distributed clinics in the USA and five in Japan participated in enrollment of patients for this study; data collection in Japan is ongoing.

Results: 129 American patient/caregiver dyads and 44 Japanese dyads completed questionnaires so far. Caregivers were similar demographically: mean age in each group was 56, 66–71% were female, about 60% had completed at least some college, close to half were working part-time or full-time.

Japanese caregivers were more likely than Americans to know patients with TIV (34% vs. 5%). Only 12% of Japanese caregivers reported having sufficient information about ALS management vs. 69% of Americans, and fewer Japanese reported excellent personal health status (16% vs. 61% of Americans).

When asked whether they had decided in favor of the patient's getting TIV, 35% of American caregivers vs. 51% of Japanese were definitely or probably in favor, 47% of Americans and 5% of Japanese were undecided, and 19% of Americans vs. 44% of Japanese were probably or definitely not in favor ($p < 0.0001$). Not only do the two groups differ, but the distributions do as well: Japanese caregivers had definite opinions, pro or con, while most Americans were undecided.

In the American sample, the most common reasons for favoring TIV included confidence in the patient's ability to maintain quality of life (83%), and the fact that the patient would live longer (61%). Only 34% were confident of family support for TIV. Too few Japanese caregivers responded to these items to meaningfully evaluate.

Reasons NOT to choose TIV were answered by only 21 American caregivers and even fewer Japanese. Most cited inevitable progression of disease and God's will as the main reasons.

Discussion: What stands out is the difference between caregiver groups regarding preference for TIV. It is surprising that so many Japanese caregivers are not in favor of TIV, given the substantially higher TIV rates in Japan to date. The modest Japanese sample size must be considered when evaluating these findings.

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P186 OPINIONS AND BEHAVIORS OF JAPANESE AND AMERICAN PATIENTS REGARDING TRACHEOSTOMY WITH INVASIVE VENTILATION (TIV)

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Keywords: tracheostomy, patient decision-making

Background: International studies report widely disparate rates of TIV between countries, between areas within countries, and even in the same hospital. When TIV is initiated, it is often done emergently without advance directives.

Objectives: We conducted surveys of American and Japanese patients and caregivers, selected because American patients have among the lowest, and Japanese the highest rates of TIV. Here we present selected findings from the patient survey.

Sample: Two groups of patients were included: recently diagnosed patients and those with respiratory impairment, defined as scores < 8 on the Respiratory subscale of the ALSFRS.

Methods: Eligible patients accompanied by a family caregiver were approached during routine clinic visits, asked whether they knew about TIV, and if so, were invited to participate in the surveys which were given to them to complete during the clinic visit. Five geographically distributed clinics in the USA and five in Japan participated in enrollment of patients for this study.

Results: 129 American and 44 Japanese patient/caregiver dyads participated to date. Mean age in both groups was about 63, nearly 50% were female. 75% of American patients and 48% of Japanese completed at least some college, and about one-fifth of both samples was employed. 75% of American patients and 50% of Japanese lived with their spouse.

Advance planning was limited in the American sample; 38% had signed DNR orders, and 59% had advance directives. This question was not included in the Japanese survey. American patients used many more sources of information about ALS and its management than Japanese, who largely relied on their neurologists and, to a lesser extent, clinic staff.

Significantly more Japanese patients knew a patient with TIV (23% vs. 5%, $p = .002$). When asked whether they had decided to get TIV, 18% of Americans and 17% of Japanese were definitely or probably in favor, 54% of Americans and 29% of Japanese were undecided, and 28% of American and 55% of Japanese were probably or definitely against having TIV ($p = .0037$). Most common reasons endorsed by American patients for wanting TIV included confidence in being able to maintain quality of life (81%), confidence in family support (72%), and respect for the value of life (55%). Too few Japanese responded to analyse meaningfully.

Reasons NOT to choose TIV included the belief that the disease would progress anyway (85%), they will be too disabled by then (82%), fear of losing all ability to communicate (50%), and belief that the outcome is God's will (50%).

Discussion: While approximately similar demographically, national differences were found regarding preference for TIV: while about one in five were in favor, more than half of American patients were undecided while more than half of Japanese patients were probably or definitely opposed to having TIV.

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P187 HEALTHCARE PROFESSIONALS' VIEWS ABOUT THE PROVISION OF GASTROSTOMY AND NON-INVASIVE VENTILATION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: healthcare professionals, gastrostomy, non-invasive ventilation

Background: Prognosis following diagnosis of ALS is poor, with survival limited to ~3–5 years from diagnosis. Current interventions tend to be limited to slowing disease progression. Bulbar symptoms commonly result in dysphagia, weight loss and respiratory muscle weakness, for which gastrostomy and non-invasive ventilation may be options. It is generally felt that despite the provision of guidelines about when gastrostomy or non-invasive ventilation (NIV) might be made available to patients, fewer patients avail themselves of these interventions than might benefit from them. It is unclear whether the availability of interventions may depend to some extent on the attitudes of healthcare professionals (HCPs) who are in a position to discuss these interventions with patients.

Objectives: To quantify the views of healthcare professionals with respect to the provision of gastrostomy and NIV for people with ALS in England, Wales and N. Ireland.

Methods: We conducted an online survey of the views of healthcare professionals about the provision of gastrostomy and NIV in ALS. Emails inviting professionals to participate were sent to: 33 Neurologists; 18 MND specialist care centres; the Association for Palliative Medicine; and the South-East MND network. There were two versions of the questionnaire, one about gastrostomy and one about NIV. Participants were asked to complete the version concerning the intervention about which they felt they had most experience. If they felt knowledgeable about both interventions, they could complete both versions.

Results: Of the 1298 healthcare professionals who received the questionnaire, 166 completed at least one questionnaire giving a response rate of 12.8%. Chi square analyses found significant differences between medical and non-medical professionals' views on: whether HCPs adhered to policy and legal constraints when it came to making gastrostomy available for people with ALS; in their impressions of patients' and carers' understanding of the effects on gastrostomy and NIV on symptoms and quality of life; and the challenge faced by HCPs when subsequently caring for patients who have refused gastrostomy. However, HCPs with more than 10 years' experience did not find this challenging.

Discussion and conclusions: Despite our low response rate, it is possible that different types of HCPs hold dissimilar views on the provision of gastrostomy and NIV. This may impact on the clinical provision of these interventions and patients' and carers' experiences. Clinical experience may also be a relevant factor when providing care for people who refuse palliative interventions. There appears to be a need for more widely available guidelines regarding the provision of gastrostomy, along with advice on the best way to impart information to patients and carers regarding gastrostomy and NIV, to allow for a potentially more consistent approach to the care of people with ALS.

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THEME 8 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P188 EXPLORING THE IMPACT OF NON-INVASIVE VENTILATION TOWARDS THE END OF LIFE

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Keywords: non-invasive ventilation, qualitative, end of life

Background: Non-invasive ventilation (NIV) is the intervention of choice for respiratory support in patients with MND. While the benefit of NIV in terms of survival has been established, the long term use of the device particularly towards the end stage of the disease has been less explored.

Objectives: The objectives of this work were: i) To describe carer and health professional experiences of end of life care for MND patients using NIV, and ii) To identify any issues encountered using NIV at this stage with a view to enhancing end of life use of NIV for MND patients and their carers.

Methods: The study used a longitudinal mixed method approach including interviews with patients, their carers and health care professionals over the course of the disease. The data reported in this paper focus on the interviews with carers and health professionals following end of life.

Results: Nine carers and 15 health professionals (representing seven disciplines) were interviewed. Seven of the patients were using NIV regularly towards the end of life, with two dying in hospital without the system in use and five having NIV in 24 hour operation at the point of death. Death for all patients was described as peaceful, regardless of whether using NIV at the time or not. Participants reported surprise regarding the rapidity of the final phase, and described when patient wishes regarding place of death or non-resuscitation had differed from the eventual circumstances. Professionals described discussion regarding "turning the machine down" or removing the mask, and raised concerns regarding difficulties in timing discussion relating to advance directives, and also in ensuring emergency services followed patient wishes/needs.

Discussion and conclusions: The major finding was that death for the majority of patients using NIV, as for patients not using NIV, was peaceful. In a small number of cases, concerns regarding impaired communication and mouthcare were raised but in the main the use of NIV did not have an adverse impact on the end of life phase of MND. NIV in the end stages was often used to comfort and ease anxiety. The majority of patients died in the community and it was striking how little medical professionals were involved in the end of life, with decisions regarding NIV being made by non-medical community teams. This indicates the importance of disseminating the end of life wishes regarding withdrawal or continued usage of NIV and any advance directives. Often, in spite of careful planning patients can still end up being taken to acute facilities at the end of life and there is a need for carers to have an alternative to calling emergency services in the final phase.

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P189 WITHDRAWING NON-INVASIVE VENTILATION (NIV) AT THE REQUEST OF A PATIENT WITH MND: THE EXPERIENCES OF DOCTORS IN THE UK

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Keywords: ventilation, withdrawal of treatment, doctors experiences

Background: Very little is known about the withdrawal of NIV at the request of a patient who has become dependent on it; in part because this is quite a rare occurrence. Exploration of the ethical context emphasises the stressful nature of this area of clinical care and how difference in opinion and belief, despite a uniform legal context, can influence a doctors practice and level of distress. The NICE guidance in England and Wales on the use of NIV in MND (2010) identifies the lack of any clarity in the most effective and acceptable method of withdrawal and how this process should be facilitated and managed. Specifically NICE suggests interviewing the professionals involved in such events as a focus of research.

Objectives: To discover the challenges to practice; to identify perceptions of the best experience for patients and families; to understand how involvement in withdrawing NIV can affect doctors.

Methods: A qualitative, exploratory approach using one-to-one in-depth interviews with doctors who have been involved in withdrawing NIV at a patient's request within the last five years. Doctors were asked to tell the narrative of cases they recalled. Interview transcripts were analyzed thematically using a grounded theory approach.

Results: 18 doctors participated from the specialties of palliative medicine, neurology, family (GP) and respiratory medicine. The bulk of direct involvement in withdrawal was undertaken within palliative medicine but other specialists were involved in decision-making and clinical advice on the withdrawal process.

Cases were few but extraordinarily memorable with explicit detail of aspects recalled from several years. The emotionality and the tensions of the situation were especially vivid whereas the logistics were more variably recalled. Doctors carried these experiences with them. Few had opportunity to share this area of practice, their experience and persisting reflections with colleagues. The clarity of the ethical and clinical decision making was in contrast to the multilayered and conflicting feelings doctors experienced in carrying out the patient's wishes. Medical indemnity organizations appeared unclear about the professional and legal acceptability of this area of care and this increased the complexity and the stress of the situations. Some colleagues also were less comfortable with the process. The withdrawing of NIV felt different to stopping other treatments notably because of the planned nature and the relatively quick time between withdrawal and death.

Discussion: This is a lonely and uncomfortable experience for doctors. The absence of guidance both practical and professional was a strong feature. There is a need to build consensus amongst those involved in discussions as well as actual withdrawal of NIV.

Conclusions The withdrawal of NIV is an area of care with a high impact on doctors. This study has identified some ways that would improve support and practice.

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P190 ANALYSIS OF THE EXPERIENCES OF PATIENTS WITH MND AND THEIR CARERS FOLLOWING THE RECOMMENDATION TO USE NIV: FACILITATING ACCEPTANCE AND TOLERANCE OF NIV

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Keywords: non-invasive ventilation, qualitative, patient and carer views

Background: Ventilatory assistance is the mainstay of respiratory support in patients with motor neurone disease (MND), with non-invasive ventilation (NIV) the intervention of choice. A recent survey in the United Kingdom (UK) reported a 3.4 fold increase in the number of MND patients who were using NIV, indicating a substantial change in practice since 2000. Use of NIV for more than four hours per day can significantly prolong survival, preserve respiratory function and maintain quality of life (QoL). While the benefit of NIV in terms of survival has been established, the process of introduction, acceptance and long term use of the device has been less explored.

Objectives: The objectives of this qualitative study were to: i) Examine the perceptions and experiences of patients with MND and their carers following the recommendation to use NIV; ii) Identify and describe factors impacting on acceptance and tolerance, and iii) Develop recommendations that may result in enhanced compliance and an improved patient and carer experience of NIV.

Methods: The work used a mixed-method approach including qualitative interviews with patients and carers within one month of NIV being initiated.

Results: The study identified themes relating to: first impressions of the technology; issues of sleep disturbance; adverse sensations of pressure and pulsing; dry mouth; design of the mask; and fitting the mask. Patients/carers perceived benefits related to: increased energy; improved sleeping; enhanced carer wellbeing; improved breathing and increased speech clarity. Bulbar dysfunction at the time of NIV initiation did not adversely affect patient compliance with NIV.

Conclusions: A key factor described by patients and carers was the need to persevere to overcome the challenges associated with early NIV use. The study highlights the importance of patient perceptions of gains as a factor in their NIV usage decisions. Key recommendations are: availability of easily accessible in-person support for patients; for clinicians to pre-empt potential obstacles by discussing options such as humidification or alternative mask interfaces; the importance of discussing potential benefits in detail with patients; patients with bulbar dysfunction should be given a trial of NIV if indicated; and optimisation of secretion management prior to the NIV trial.

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P191 ASSISTED VENTILATION DEPLOYMENT IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS AT CAROLINAS NEUROMUSCULAR/ ALS-MDA CENTER: PRESSURE-BASED (P-) AND VOLUME–BASED (V-) NON-INVASIVE VENTILATION (NIV) COMPARED WITH TRACHEOSTOMY-PERMANENT VENTILATION (TPV) – A 10-YEAR EXPERIENCE

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Keywords: pressure-based ventilation, volume-based ventilation, tracheostomy-based ventilation

Background: Increased clinical use of NIV has become widespread (1). In the USA, NIV deployment before the 1999 AAN ALS Guidelines was 8%-9%(2,3) but rose to 15.6%(2000) (4) then 21%(2004)(3) and subsequently increased to 36%(2006) but this was felt to still be below the guideline recommendations (5). In the United Kingdom, NIV use has increased from 13.6% (2000) to 36.1%(2009) based on two national surveys(6,7). A population-based study in northern Italy identified NIV use at 20.6% (2004) while published center-based studies identified deployment five years later at 37%(2009)(8,9). NIV deployment in French ALS patients at a single center has increased from 16 % (2004) to 36 %(2006) to 51%(2008) based on increased sensitivity to respiratory insufficiency(10).

Objectives: Compare impact on survival of patients with ALS using Bi-directional Positive Airway-Pressure (BiPAP)-based P-NIV with those using volume-based V-NIV and TPV in a single-center-based clinical audit.

Methods: From 2003–2012, 387 total patient with ALS ((219-F: 168-M)) received respiratory support. Deployment of P-NIV, V-NIV and disease trajectory to death or last-follow-up was collated. Descriptive statistics of clinical features prior to respiratory intervention and changes overtime including transition from P-NIV to V-NIV to TPV were compared with survival between groups. Statistical analysis was performed with MedcCalc software(version 12. 2.1.0).

Results: From 2003–2008, 198 patients received P-NIV. From 2009–2012, 142 patients received V-NIV. From 2003–2012, 47 patients received TPV. Survival was significantly ($p = 0.017$) improved with a decreased hazard ratio = 0.732(95%CI = 0.567–0.949) in patients with V-NIV (50% survival = 15 months (95%CI = 11–18)) compared with P-NIV (50% survival = 9 months (95%CI = 8–11)). Survival was significantly ($p = 0.026$) improved with a decreased hazard ratio = 0.662(95%CI = 0.449–0.979) in patients with TPV (50% survival = 22 months (95%CI = 12–35)) compared with V-NIV. There was no difference in the use of riluzole across the groups.

Conclusions: Deployment of V-NIV is associated with a mean improved survival of 6 months relative to P-NIV. TPV still has the most dramatic effect with a further mean increased survival of 22 months among patients with ALS, even when deployed following NIV.

Discussion: Further single center-based audits of P-NIV compared with V-NIV and TPV will be required to assess the relative potential impact of these interventions on survival and quality of life in patients with progressive respiratory insufficiency.

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P192 EXCESSIVE DAYTIME SLEEPINESS DOES NOT PREDICT THE DEGREE OF SLEEP-DISORDERED BREATHING IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): AN AUTONOMIC DYSFUNCTION MATRIX

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Keywords: disordered sleep, autonomic nervous system, peripheral autnomic tonometry

Background: Although fatigue is a common symptom in amyotrophic lateral sclerosis (ALS), excessive daytime sleepiness (EDS) is not commonly reported by patients or caregivers. ALS patients with sleep disordered breathing (SDB) are less sleepy than Non ALS patients with SDB. In ALS, the degree of EDS is also not related to the Apnea Hypopnea Index (AHI) or O_2 desaturation unlike the non ALS population with SDB. As the sympathetic nervous system plays a critical role in alertness, we hypothesized that in ALS patients with SDB, the degree of subjective EDS cannot predict the degree of SDB because of autonomic dysfunction seen in ALS.

Objectives: To correlate the degree of EDS in ALS patients with underlying degree of SDB as a measure of autonomic dysfunction.

Programme description: Consecutive newly diagnosed ALS (24) patients were studied over 6 months. ALS symptom onset, Sitting and supine FVC (Forced Vital Capacity), NIF (Negative Inspiratory Force), Daytime resting O_2 saturation as well as daytime sleepiness assessed by Epworth Sleepiness Scale (ESS) were recorded. These patients also underwent an unattended home based sleep study by WatchPAT 100, Itamar Medical with AHI, RDI (Respiratory Disturbance Index) and ODI (Oxygen Desaturation Index) calculations.

Clinical outcomes: Out of 24 patients one did not complete ESS. Total 23 patients with mean ESS of 7 were divided into two groups based on the ESS score: a less sleepy group with ESS score < 10 ($n = 16$) and a sleepier group with ESS score ≥ 10 ($n = 7$). Less sleepy group had mean ESS of 4.3 ± 2.58 vs. sleepier group with mean ESS of 13.3 ± 4.7, $p < 0.0001$. Less sleepy group had mean AHI of 15.97 ($\pm 16.$) denoting a moderate degree of SDB; while more sleepy group had mean AHI of 12.18 (± 9.9); suggestive of a milder degree of SDB and poor correlation between these two parameters with $P > = 0.05$ by Mann-Whitney U Test. Overall, the less sleepy group had a higher AHI (15.97 ± 16.69 vs. 12.18 ± 9.89) than the sleepier group. Out of the 23 patients, the highest AHI of 54.1 was associated with ESS of only 3 while highest ESS of 23 was associated with an AHI of 10.4.

Recommendations to the field: In ALS patients with SDB, the degree of subjective daytime sleepiness is not related to degree of AHI and underlying SDB. This can be secondary to dysfunction of central adrenergic alerting mechanisms. As autonomic dysfunction is common in ALS, these findings help to explain the lack of daytime hypersomnolence. Further studies to look at autonomic function testing should be considered in ALS patients.

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P193 A COMPARISON OF RESTING ENERGY EXPENDITURE WITH PREDICTIVE EQUATIONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: indirect calorimetry, predictive equations, nutrition

Background: Nutrition is a prognostic factor for survival in ALS. Previous research has demonstrated an increase in caloric needs (1). However, the standard equations that estimate Resting Energy Expenditure (REE) are not as reliable in ALS patients as in the normal population (2). Therefore, a method to estimate REE accurately while the patient is in ALS clinic is needed.

Objectives: There were two objectives to our study. First, we needed to know as accurately as possible the REE in a large cross-section of ALS patients while the patient was at a routine clinic visit. Second, we compared that measurement against four standard predictive equations (i.e. Mifflin-St Jeor, Harris Benedict, Ireton-Jones, and calories per kilogram body weight) so that we might estimate caloric needs in a simpler and more accurate manner.

Methods: A chart review was performed on 143 patients (77 male/66 female) with ALS who were measured for REE using indirect calorimetry (KORR MetaCheck Metabolic Analyzer Model 7100.). All patients were instructed to fast for 4 hours, avoid all caffeinated beverages, and avoid exercise on the day of the MetaCheck. Patients were seated at rest for 15 minutes prior to the procedure. The test was adapted to use a face mask rather than the provided mouthpiece to ensure an adequate mouth seal. The testing time was 10 minutes of breathing at a normal rate through the face mask. Measured REE was compared to calculations from the Mifflin-St Jeor, Harris Benedict, and Ireton-Jones equations in addition to calories per kilogram body weight.

Results: 137 of the 143 patients were able to complete the MetaCheck analysis. Six were excluded due to low tidal volumes. Data has been collected and results are pending analysis.

Discussion and conclusion: Pending analysis of results.

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P194 HYPERCARBIA IN ALS/MND PATIENTS: UNDER-RECOGNIZED AND UNDER-DIAGNOSED-YET ASSESSING CAN CHANGE TREATMENT ALGORITHMS AND IMPROVE QUALITY OF LIFE

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Keywords: hypercarbia, hypoventilaton, diaphragm pacing

Background: Patients with ALS/MND suffer from hypoventilation and can suffer from the effects of hypercarbia which can lead to headaches, cognitive impairments and decreased survival. In many patients hypercarbia will precede any evidence of hypoxia. Increasing knowledge of developing hypoventilation and hypercarbia will help identify patients before acute respiratory failure.

Objective: Identify the incidence of hypercarbia in ALS/MND patients during presentation with routine utilization of arterial blood gases for analysis of carbon dioxide levels (CO_2).

Methods: All arterial blood gas results were prospectively obtained at one institution during respiratory evaluation in an ALS/MND clinic under local IRB protocols and a retrospective analysis of prospective data in the pivotal FDA trial of diaphragm pacing for ALS at multiple sites.

Results: An analysis of the database of 148 ALS patients arterial blood gases obtained on initial evaluation were analyzed and 44% of patients presenting had an elevated CO_2 level. 25% of subjects had a severely elevated pCO_2 and none of them had a previous evaluation for hypercarbia. In the diaphragm pacing multi-centre pivotal trial paired sample analysis (74 subjects) there was a decrease of 2.0 mmHg of CO_2 with utilization of diaphragm pacing ($p < 0.001$). In patients with severely elevated pCO_2in the diaphragm pacing study (18 subjects) there was a decrease of 2.6mmHg of CO_2 ($p < 0.03$) with use of diaphragm pacing. In clinical practice the largest change of $pC0_2$ was from 54 to 40 with diaphragm pacing alone and in this patient there was no utilization of non-invasive ventilation (NIV).

Conclusion and discussion: This evaluation is limited since the ALS subjects evaluated were being assessed at research centres involved in therapies for diaphragm dysfunction so the results may not reflect the global ALS/MND population but the incidence of hypercarbia is still significant and represents a large subset. Hypercarbia can be unrecognized and is an ominous predictor of acute respiratory failure and death. Assessment should become a standard in ALS/MND management. Future assessment with short term awake supine capnography or transcutaneous CO_2 techniques may alleviate arterial blood gases but these techniques need further evaluation before they can replace arterial blood gases. Once identified, hypoventilation and hypercarbia can be treated with either increasing use of NIV or diaphragm pacing if the patient has a stimulatable diaphragm. Early detection and knowledge can allow utilization of available therapies to slow the progression of hypoventilation and improve the patients' quality of life by decreasing the adverse effect of an elevated CO_2 and its systemic effects which includes cognitive dysfunction.

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P195 EFFECTS OF ENTERAL NUTRITION ON SURVIVAL IN BULBAR VERSUS SPINAL ONSET ALS PATIENTS

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Keywords: enteral nutrition, survival, bulbar

Background: Studies investigating the survival effects of enteral nutrition (EN) by percutaneous endoscopic or radiological gastrostomy (PEG or PRG) in ALS have shown mixed results suggesting that patient selection may be an important factor.

Objective: The purpose of this study is to begin to assess the effects of PEG or PRG and EN on the survival time of different ALS patient populations (bulbar onset versus spinal onset).

Methods: The authors conducted a retrospective search of the UIC ALS Database. All ALS patients evaluated by the UIC ALS speech and communications team during the period of 1 December 2005 to 25 April 2012 were included in the database. 143 patients were considered for inclusion. Inclusion criteria included: 1). Definite or probable ALS at some stage of disease 2). Available date of death 3). Known spinal versus bulbar onset 4). Known diagnosis date and patient reported symptom onset date. 63 patients were included, 19 bulbar onset (13 with EN, 6 without EN) and 44 spinal onset (7 with EN and 37 without EN). EN was recommended according to the AAN practice parameter guidelines. The survival distribution of the two samples with or without PEG or PRG was evaluated using the Log-Rank test after selecting for bulbar and limb onset as a sub-group analysis. In addition, a survival comparison was conducted for the sub-groups receiving riluzole and/or BiPAP.

Results: For bulbar-onset patients receiving EN, the median length of survival from symptom onset and from diagnosis was 973 and 707 respectively, and for those who refused EN was 524 and 196 days respectively. The distribution differences in survival from symptom onset and from diagnosis between the EN and no EN groups were both statistically significant, $p = 0.014$, and $p = 0.019$. For spinal-onset patients receiving EN the median length of survival from symptom onset and from diagnosis was 843 and 504 days respectively, and for those who refused EN was 722 and 459 days respectively. The survival distributions from symptom onset and from diagnosis were not significantly different between the EN and no EN groups. Demographic characteristics between the two samples (with or without PEG/PRG) were no different for age, gender and race. There was also no difference in BiPAP or riluzole use.

Discussion: In our ALS patient population, bulbar-onset patients (progressive bulbar palsy) who received EN either by PEG or PRG had a statistically significant improvement in survival as opposed to spinal-onset patients who did not.

Conclusion: Localization of symptom onset may be an important factor in patient selection for PEG or PRG. Further study is needed to confirm these findings in a larger patient population and to identify additional factors impacting upon survival in ALS patients considered for EN.

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P196 ALS DASHBOARD STAGING SYSTEM BULBAR AND RESPIRATORY DOMAIN MILESTONES ARE SIMILAR IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS): TIME FROM GASTROSTOMY TUBE (GT) TO NON-INVASIVE VENTILATION (NIV) IS SIMILAR TO TIME FROM NIV TO GT

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Keywords: disease staging, benchmarking, ALS Dashboard

Background: Gastrostomy tube (GT) placement and initiation of non-invasive ventilation are employed to improve nutrition and respiration in patients with ALS. Both interventions occur at different times in various patients and each constitute a definite milestone in the ALS Dashboard staging system being evaluated to assess domain (bulbar, respiratory) stage specific disease progression.

Objectives: Compare disease trajectory for patients with ALS who receive GT before NIV or NIV before GT in a single-center-based clinical audit.

Methods: From 2007–2012, 35 patients (19 F;16M) received GT before NIV and 46 patients (24M; 22 F) received NIV before GT. Disease trajectory was assessed by ALSFRS-R and ALS Dashboard. Weight, body mass index at time of GT insertion, change in weight and body-mass index prior to GT insertion and following GT insertion, vital capacity at initiation of GT and at initiation of NIV, change in vital capacity pre-GT and pre-NIV, change in vital capacity post-GT and post NIV, survival post GT followed by NIV and post NIV followed by GT were analyzed. Statistical analysis was performed with MedcCalc software(version 12. 2.1.0).

Results: Surprisingly, time from GT to NIV in 35 patients with ALS who had bulbar disease domain progression first (mean = 9.6 months; 95%CI = 5.9–13.8 months) was identical to time from NIV to GT in 46 patients with ALS who had respiratory disease domain progression first (mean = 9.8 months 95% CI = 6.1–14.4 months). Overall survival in each group was comparable regardless of the initial intervention.

Conclusions: Deployment of GT first in bulbar domain progressing patients followed by NIV or NIV first in respiratory domain progressing patients followed by GT proceeds along similar timelines.

Discussion: Changes in ALS Dashboard respiratory stage in bulbar onset/affected patients with ALS is comparable to changes in ALS Dashboard bulbar stage in respiratory onset/affected patients with ALS. ALS Dashboard change within bulbar and respiratory domains provides comparable rates of disease trajectory change over time. Further studies will be required to assess whether newer diagnostic techniques leading to earlier intervention or identifying pharmacological or

physical treatment interventions will affect these milestone changes in a similar fashion.

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P197 "WOULD YOU DO IT AGAIN?" PATIENT PERSPECTIVE REGARDING THEIR DECISION FOR PEG IN ALS

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Keywords: percutaneous endoscopic gastrostomy, decision, satisfaction

Background: Percutaneous endoscopic gastrostomy (PEG) has become the intervention of choice for ALS patients when oral food intake becomes unsafe or inadequate. But there are risks associated with the procedure and PEG does not stop disease progression. Studies have shown that PEG is probably effective in prolonging survival but this has not been quantified (AAN Practice Parameters). There has been research associated with the decision-making process but little on patient feelings about their decision after having the procedure. However, PEG continues to be recommended and many patients accept it.

Purpose: This study was to determine patient perspective regarding their decision to have a PEG after they had the procedure and started using the PEG.

Method: In the course of assessment and follow-up for each patient by the Speech Language Pathologist (SLP), Registered Dietitian (RD) and/or Clinic Nurse (RN), answers to three specific questions were recorded: 1)Are you an "eat to live" (ETL) or "live to eat" person (LTE)? 2) How did the PEG insertion procedure go and were there any complications? 3) Would you do it again? (YES or NO). Patient comments were recorded. Site of onset, indicators for PEG, PFTs, BMI (initial and a time of procedure) and survival were also recorded.

Results: Complete data for 22 patients (14 active, 8 deceased) have been collected (twelve patients with PEG but incomplete data were not included). Three patients (14%) stated that they would not "do it again". All 3 were LTE. Two had complications (pain, infection). One patient is deceased (627 days); the other two are presently living (639 and 738 days post-procedure). One patient has returned to eating orally. Of the YES group, 58% were ETL. Comments from the YES group were generally positive. One patient found it a lot better than anticipated. The most common negative comment was missing taking food orally. Additional comments were about adjustments re: body image, "medicalization" of eating and "the alternative is I would starve or choke and shorten my life".

Discussion: The result that 86% would do it again would indicate that the procedure is perceived by patients who choose to have a PEG to be a valuable component of their treatment. ETL group had more positive responses and comments. Complications did not appear to have an impact on satisfaction.

Conclusion: This study confirms our clinical observation that the majority of our patients with ALS are satisfied with their decision to get a PEG. Comments from both groups provided insight into this aspect of patient care. The result that even patients who would "do it again" had negative comments indicates that continued support of all patients post-procedure is important.

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P198 ALS BODY WEIGHT AFTER PEG PLACEMENT

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Keywords: dietician, body weight, weight loss

Background: Significant weight loss, as a result of insufficient food intake, reduces the survival rates in patients with ALS. The most recent practice parameter (1) suggests that enteral nutrition via percutaneous endoscopic gastrostomy (PEG) will stabilize body weight. Clinical data on the pattern of weight loss before and after PEG insertion, and particularly the effect of dietetic intervention, are scarce.

Objective: To determine if it is possible to stabilize body weight in ALS patients after PEG insertion.

Method: Data were retrospectively derived from the dietitians' database between 2007 and 2011. Only patients with a PEG inserted were included in the analysis. A total of 21 patients were identified as suitable. The changes in body weight (kg/month) before and after PEG insertion were compared and checked with the paired sample *t*-test.

Results: Before PEG, 16 patients lost weight and 5 gained weight: the mean weight loss was 0.8 kg/month. After PEG, 14 patients lost weight, 2 stabilized and 5 others gained weight: the mean weight loss was 0.2 kg/month. The reduction in weight loss before and after PEG was not significant ($p = 0.069$). Patients with spinal onset ($n = 15$) had a mean weight loss of 0.7 kg/month before and stabilized after PEG insertion. Patients with bulbar onset ($n = 6$) had a mean weight loss of 0.8 kg/month before and 0.3 kg/month after PEG insertion. Weight loss after PEG was caused by a decreased energy intake.

Discussion: There is little evidence (1), that body weight stabilizes after PEG insertion. The present study indeed shows that body weight continues to drop after PEG placement, albeit in a reduced pace. After PEG insertion, 5 patients temporarily gained weight (mean 2.3 kg), but later they lost weight again.

We found no studies which took into account the wishes of the patient when deciding treatment, such as when to start or increase enteral nutrition, or to use a feeding pump when enteral nutrition by bolus is no longer tolerated. Also, close monitoring by a dietitian can prevent severe weight loss in ALS patients before PEG insertion.

Conclusions: Our study shows that after PEG insertion, weight loss might be minimized by various interventions by

the dietitian. These interventions can also play a significant role in weight control before insertion. Ultimately, it is the patient who should decide if and when any nutritional interventions are implemented. More studies are needed to show which factors that are amenable to intervention determine weight loss in ALS patients after PEG placement.

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P199 EARLY LOSS OF BODYWEIGHT IN ALS – IMPORTANT FOR DISEASE PROGRESSION?

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Keywords: nutrition, bodyweight, ALSFRS

Background: Reduced ability to maintain nutritional status is common among patients with ALS. During the disease progression and when bulbar symptoms arise it is difficult for the patients to maintain a balance in the calorie intake. Malnutrition leads to impaired muscle strength both in extremities and respiratory muscles and has a negative effect on the patient's quality of life. Body weight and BMI is simple methods to measure the nutritional status and is commonly used in most ALS team.

Object: The aim was to evaluate if the patients nutritional status, measured by body weight and body mass index (BMI), has an effect on the disease progression, measured by ALSFRS.

Methods: This is a retrospective study where the data were collected by record review. A total of 35 patients were included, 18 patients with PEG and 17 without PEG. At each patients record there were data on weight, height and ALS FRS at a minimum of three time points.

Results: In the group with PEG, weight loss before diagnosis was 7% in spite of where the symptoms started, with a mean BMI of 24.5 (SD 2.9). During the time between diagnosis and PEG the weight loss continued with a total weight loss of 10%. A stabilisation of body weight came after three months. ALSFRS at time for diagnosis was 32/40 and at the time for PEG 26/40. Three months after the PEG had been inserted the ALSFRS value was 21/40 but then the progression was slower. In the group without PEG treatment the weight loss was 5% before diagnosis with a BMI of 25.3 (SD 3.1). Their weight loss continued one year after time for diagnosis and then stabilized. At diagnosis this group had an ALSFRS value of 35/40 and after one year 28/40.

Discussion: None of the patients in this study had a BMI < 21. BMI alone was not a good indicator of malnutrition. Since this was a study with a longitudinal approach, patients died during the follow up period which complicated analysis of data. However, it is important to evaluate the change in total weight loss and not just current body weight and BMI.

Conclusion: Of importance was the weight loss before diagnosis. When the bodyweight was stabilized the disease progression slowed down as measured with ALSFRS.

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THEME 9 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P200 THE EDINBURGH COGNITIVE ALS SCREEN (ECAS)

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Keywords: cognition, behaviour, screen

Background: A frontotemporal syndrome occurs in up to 50% of people with ALS. However the cognitive status of the majority remains unknown due to lack of resources and of appropriate screening tools. Here we present the Edinburgh Cognitive ALS screen (ECAS), a 15 minute standardised interview for use by health care professionals in the clinic. The ECAS was developed to be sensitive and specific to cognitive and behavioural changes in ALS and ALS-FTD. The ECAS is unique in being designed; a) specifically for ALS patients with varying motor disability, b) to be sensitive to the range of impairment in ALS including both executive and language dysfunction, c) to distinguish ALS with cognitive impairment from prodromal Alzheimer's Disease and other conditions common in older adults.

Objectives: To demonstrate sensitivity of ECAS to cognitive and behavioural change in ALS.

Methods: 23 non-demented patients with ALS were compared with 20 healthy controls, closely matched for age and years of education. In addition, two cases of ALS-FTD were tested. All participants were interviewed by a nurse specialist. The ECAS is a multidomain interview with sections assessing ALS-specific functions (executive and language) and ALS-nonspecific functions (memory, visuospatial) and a separate carer behaviour interview based on new criteria for diagnosis of behavioural variant FTD.

Results: a) ALS-non demented patients. ALS-specific functions: In total 43% (10) patients showed impairment with 26% (6) a verbal fluency deficit, 22% (5) general executive dysfunction and 26% (6) language dysfunction (spelling impairment). ALS-nonspecific functions: No patients showed a deficit on visuospatial functions. 9% (2) showed memory retention deficit with only, one showing problems with ALS-specific function. Behaviour: 21% of patient carers described changes in behaviour with 17% Apathy, 4% (1 patient) Perseveration and Stereotyped behaviour and 4% (1 patient) with Hyperorality and change in Eating Behaviour. Of note 9% (2 patients) showed behaviour changes without cognitive dysfunction. b) ALS-FTD. ALS-specific functions: the patients were impaired across all elements with prominent executive and spelling dysfunction. ALS-non-specific functions: neither patient was impaired on memory retention. One patient showed poor visuospatial performance. Behaviour: Both patients showed predominant behaviour dysfunction across components.

Discussion and conclusions: This study demonstrates that the ECAS is a sensitive measure of the frontotemporal syndrome in ALS and ALS-FTD. The proportion of non-demented patients detected with cognitive impairment was in line with epidemiological research. Moreover patients showed the pattern of deficits, which is specific to the disease. Of note, one patient showed an independent ALS-nonspecific memory retention problem and it is predicted that this patient has co-morbid prodromal Alzheimer's disease. Further longitudinal and neuropathological findings may provide evidence for this. Future studies will continue to validate this screen.

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P201 EXECUTIVE DYSFUNCTION IN ALS RELATES TO REDUCED WHITE MATTER INTEGRITY IN THE FRONTAL LOBES

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Keywords: cognition, correlation, white-matter

Background: Cognitive impairments are being increasingly recognised as part of the heterogeneous presentation of ALS patients. Deficits in tests of executive functions are the most commonly reported impairment in ALS, however, slowed processing speed, a predominant feature of other motor disorders, has received little attention. Reduced white matter integrity has been shown in tracts and cerebral regions associated with extra-motor functioning, however, direct correlations with cognitive performance are rarely undertaken.

Objectives: This study aimed to determine whether a) cognitive impairments observed in ALS are underpinned by executive dysfunction or slowed processing speed, and b) to identify the loci of white matter changes which may underpin any observed impairments.

Method: Cognitive functioning was investigated in 30 ALS patients and 30 age and IQ-matched controls using tasks designed to account for motor disability. Novel dual-task and processing speed paradigms were designed specifically to test the competing theories of executive functioning and speed. Background neuropsychological tests were also administered. In addition, diffusion tensor magnetic resonance imaging (DTI) data was obtained, allowing measurement of white matter integrity in anterior and posterior cerebral tracts through region of interest measures of fractional anisotropy and mean diffusivity.

Results: ALS patients performed significantly worse than controls in the dual-task and letter fluency test. However, the ALS patients performed comparably to controls on tests

of processing speed. Patients' performance in the dual-task correlated with white matter integrity in the association fibres adjacent to dorsolateral prefrontal cortex. Patients' performance in letter fluency correlated with white matter integrity in the Genu, and association fibres adjacent to Broca's area and Broadman's area 10.

Discussion and conclusions: ALS patients were impaired on tests of executive functioning, whilst showing intact processing speed. Performance in the dual task and letter fluency test was associated with dysfunction to different pathways within the prefrontal cortex. The ALS group exhibited cognitive impairments indicative of executive dysfunction which were associated with extensive structural white matter changes in the frontal lobes. The investigation of white matter integrity through DTI is a sensitive method for revealing extra-motor changes and heterogeneity within ALS populations.

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P202 CORTICAL THICKNESS CHANGES IN ALS WITH OR WITHOUT COGNITIVE AND BEHAVIOR DISTURBANCES COMBINED WITH NEUROPSYCHOLOGICAL ASSESSMENTS

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Keywords: cortical thickness, neuropsychological assessments, cognitive impairment

Background: Some amyotrophic lateral sclerosis (ALS) patients have apparent cognitive and behavior disturbances which meet the criteria of ALS-FTD, and some only have mild symptoms named ALSci or ALSbi. Sensitive neuropsychological examinations can be used for cognitive assessment, and cortical thickness measurement used for learning the structural features.

Objectives: The aim of the study is to discuss the cortical thickness changes in ALS with and without cognitive and behavior disturbances, and find whether there is any correlation between cortical thickness and neuropsychological examinations.

Method: Cortical thickness measurements were performed on structural 3T MRI data of 18 patients and 18 matched healthy controls. Patients would have had tests of *verbal* fluency, prospective memory, theory of mind and picture emotional perception. Patients were divided into pure ALS, ALSci and ALS-FTD according to the tests and symptoms. Cortical thickness was analysed separately, and the correlation was checked with cognitive examinations.

Result: Cortical thickness of whole brain 70 regions showed no difference between pure ALS patients and controls ($P > 0.05$). Cortical thickness of bilateral lateral orbitofrontal, bilateral entorhinal, left medial orbitofrontal, and left parsorbitalis cortex were decrease in ALSci. Diffused reduction of cortical thickness was observed in bilateral hemisphere in ALS-FTD. Positive correlations were found between EBPM, TOM, arousal of negative pictures, recognition of pictures and their corresponding brain regions ($P < 0.05$) .

Discussion: Gliosis might counteract the neuron shrinking, and would lead to no cortical thickness reduction of precentral cortex in pure ALS. Atrophic regions in ALSci could be explained by the cognitive impairments found in patients (using neuropsychological examinations), and we supposed they might relate to the deposition of pThr175-tau. Cognitive and behavior disturbances might be the reason of cortical thickness changes in extra-motor regions, since it was normal in pure ALS. The correlations between neuropsychological examinations and brain regions could all be explained by their anatomic and functional basis. Therefore, we consider that cortical thickness measurements could be used as a sensitive tool to reflect the structural change of cognitive impairment.

Conclusion: Cortical thickness in precentral cortex of ALS patient was not always decreased. Changes of cortical thickness showed a greater difference in pure ALS, ALSci and ALS-FTD, which would help to find the nature of disease. Some neuropsychological examinations had significant relationship with their corresponding brain regions. For those with mild cognitive impairment, cortical thickness measurements could be a sensitive way to assess the cortical structure in an early stage.

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P203 SOURCE ANALYSIS OF COGNITIVE FUNCTIONS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS BY MEANS OF AUDITORY P300 ODDBALL PARADIGM

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Keywords: event related potentials, cognitive impairments, source analysis

Background: Traditionally, ALS is considered as a disorder limited to motor neurons in the primary motor cortex, in the brainstem and in the spinal cord, although in recent years, cognitive deficit has been demonstrated in non-demented patients with ALS. Current research has emphasized the usefulness of Event Related Potentials (ERPs) to assess subclinical cognitive impairment. P300 (P3) is the most studied cerebral wave in evaluating cerebral information processing during the course of this neurological disease and several electrophysiological studies provide evidence for extra-motor areas, demonstrating sub-clinical cognitive deficits in patients with ALS.

Objectives: To determine and localize sources of electrical cognitive activities using a P3 auditory stimulation "oddball" paradigm and to investigate the presence of sub-clinical cognitive dysfunction in ALS patients.

Methods: BAEPs were recorded for hearing assessment. P3 component was also recorded in 15 patients with ALS according to a paradigm validated in 25 healthy volunteers

using 32 EEG channels. The amplitude and latency for the component obtained were measured. The P3 component was obtained by subtracting the response to the frequent stimuli from that to the infrequent stimuli. P3 Grand Average was computed and electrical sources were estimated by means of Bayesian Model Averaging (BMA) approach in all groups. Clinical variables, depression and cognitive status were also evaluated with standardized scales for all groups.

Results: Normal BAEPs were present in all groups. P3 was not significantly different between patients and healthy controls for latencies, while the amplitude obtained was lower in patients than in controls. In the patient group, P3 amplitude correlated with age of disease onset, time of disease evolution and Beck depression inventory, but it was not related with other severity variables of disease. Significantly lower activation was also found at the level of source generators (postcentral gyrus, superior parietal gyrus, inferior parietal gyrus, and supramarginal gyrus of both hemispheres) in ALS patients as compared with healthy controls.

Conclusions: Decreased P3 amplitude indicates the presence of sub-clinical cognitive deficits in ALS patients. ERPs source analysis is a useful tool in investigating neural substrates and cerebral regions involved in specific cognitive functions. ERPs represent a convenient technique to detect and assess sub-clinical impairment in patients with severe loss of motor function. Our findings confirm the hypothesis of a sub-clinical cognitive impairment in ALS patients, suggesting pathological involvement beyond the motor areas.

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P204 THE ACCURACY OF THE MINI MENTAL STATE EXAM IN DETECTING FRONTAL TEMPORAL COGNITIVE IMPAIRMENT IN MOTOR NEURON DISEASE

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Keywords: cognitive screening, diagnostic validity

Background: The Mini Mental State Exam (MMSE) is used routinely in clinical practice and research of patients with motor neuron disease (MND) despite recognized limitations for evaluating frontal temporal cognitive impairment common in MND. The ALS Cognitive Behavioral Screen (ALS-CBS) was developed specifically for screening frontal temporal cognitive impairments in MND and has published diagnostic validity data for examining such symptoms in MND. The diagnostic accuracy of the MMSE and a MND-specific screening tool has never been directly compared in a sample of patients with MND.

Objectives: To compare the diagnostic accuracy, sensitivity, and specificity of the MMSE and the ALS-CBS in detecting frontal temporal impairment in MND.

Methods: Fifty patients with MND were administered the MMSE, the ALS-CBS-Cognitive Scale, and an independent comprehensive neuropsychological evaluation. Neuropsychological evaluation findings were used as the gold standard for classifying patients into 3 diagnostic groups: Normal, Mild Cognitive Impairment (MCI), and Dementia. Using accepted cut-off scores for the MMSE and ALS-CBS, diagnostic accuracy of the screening measures was evaluated in distinguishing between patient groups.

Results: Neuropsychological evaluation yielded 10 Normal, 19 MCI, and 21 Dementia cases. Kruskal-Wallis and Mann Whitney U tests revealed no significant group differences in patient demographics or disease characteristics. Kruskal-Wallis tests revealed predicted group effects for MMSE (χ^2 (2, $N = 50$) = 24.9, $p < 0.0001$) and ALS-CBS-Cognitive Scale (χ^2 (2, $N = 50$) = 34.0, $p < 0.0001$). Scores were: MMSE (Normal (median = 29.1; IQR 28.6, 29.3)); MCI (median = 27.9; IQR 26.1, 27.9); Dementia (median = 24.0; IQR 20.6, 26.1)] and ALS-CBS-Cognitive Scale (Normal (median = 16; IQR 15, 17.3); MCI (median = 12; IQR 10, 14); ementia (median = 7; IQR 4.5, 8)). In distinguishing Normal from Dementia patients using accepted cut-off scores for MMSE (< 23) and ALS-CBS-Cognitive Scale (< /= 10) the MMSE had a diagnostic accuracy of 58% (Sensitivity = 38%; Specificity = 100%) compared to 90% for the ALS-CBS-Cognitive Scale (Sensitivity = 86%; Specificity = 100%). In distinguishing Normal from MCI/Dementia patients, using accepted cut-off scores for MMSE (< 26) and ALS-CBS-Cognitive Scale (< 17) the MMSE correctly identified 58% (Sensitivity = 48%; Specificity = 100%) compared to 86% with the ALS-CBS-Cognitive Scale (Sensitivity = 98%; Specificity = 40%). Uniformly, false negative screening errors were more common using the MMSE than the ALS-CBS Cognitive Scale.

Discussion: The MMSE has limited accuracy in detecting frontal temporal cognitive impairment in MND. Using the MMSE for cognitive screening in MND may inflate false negative screening errors and consequently preclude early identification of MND patients with cognitive impairment. Findings question the validity and utility of the MMSE as a cognitive screening tool for patients with MND and support the use of screening tools designed and specifically validated for use in MND.

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P205 MEMORY FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS IN COMPARISON TO PATIENTS WITH MILD COGNITIVE IMPAIRMENT: A NEUROPSYCHOLOGICAL AND STRUCTURAL ANALYSIS

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Keywords: memory functions, cognition, structural MRI

Background: Cognitive functions have been found to be deficient in patients with amyotrophic lateral sclerosis (ALS), but unlike in patients with mild cognitive impairment, where temporal dysfunctions are assumed to cause the deficits, the role and characteristics of memory impairment in ALS remain unclear.

Objectives: Define and compare memory functions in ALS patients with a group of amnestic MCI patients (aMCI)

using a neuropsychological assessment and structural MRI analysis.

Methods: 40 ALS-Patients, 40 amnestic MCI patients and 40 healthy age- and gender-matched controls were subjected to perform neuropsychological tests. In addition structural MRI was acquired and analyzed using voxel-based morphometry (VBM).

Results: The analysis of the verbal memory performance (measured with Auditory Verbal Learning Test) revealed the following pattern: The aMCI group showed a significantly poorer performance in the delayed verbal recall compared to ALS patients and healthy controls ($p < 0.01$). Similar effects were observed in the recognition task. Amnestic MCIs recognized a significantly lower amount of previously presented words than the group of ALS patients and healthy controls while ALS patients made more errors during the recognition. After error correction of the recognition performance, there were significant differences between the ALS group and the healthy controls ($p < 0.05$). Analyzing the structural data we found both temporal and frontal atrophies in the ALS patients in correlation with the above mentioned results.

Conclusion: Memory impairment is evident in patients with ALS. While aMCI patients suffer from a mainly hippocampal dysfunction, memory impairment in ALS patients appears to be linked to fronto-temporal dysfunctions. This is supported by neuropsychological and structural changes observed in the MRI and could be a relevant factor for the progression of the disease.

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P206 LANGUAGE IMPAIRMENT IN ALS: PREVALENCE, PROFILE AND RELATION TO EXECUTIVE DYSFUNCTION

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Keywords: cognition, executive dysfunction, language

Background: Executive dysfunction has been the most frequently investigated cognitive domain in non-demented ALS patients. Studies have frequently reported impairments in fluency, set-shifting, attention and inhibition. Recent consensus criteria(1) have defined cognitive impairment in ALS based on impaired scores ($\leq 5^{th}$ percentile of age- and education-matched controls) on at least two measures of executive functioning, although cognitive impairments observed in ALS patients are not restricted to the executive domain. Studies have reported impairments on tasks of language function including confrontation naming, single word and syntactic comprehension and verb processing. However, there have been comparatively few systematic investigations of language in non-demented ALS patients and the nature and frequency of language impairment has not been fully characterised. Where impairments have been identified, it remains unclear whether they reflect an aphasia-like impairment or a primary executive dysfunction.

Objectives: The current study aimed to investigate the prevalence and nature of language involvement in non-demented patients with ALS and examine whether language impairments can be sufficiently explained by executive dysfunction.

Method: Neuropsychological assessment data was obtained from 51 patients with ALS and 35 healthy controls matched for age, gender and IQ. Composite scores were derived for the domains of language, executive, memory and visuospatial functioning. Domain impairment was defined as a composite score $\leq 5^{th}$ percentile relative to the control mean.

Results: Analysis revealed that the ALS patients demonstrated significant impairments in domains of executive and language functioning compared to healthy control participants, with significantly lower composite scores indicating poorer performance. Language impairment was found in 43% of ALS patients, and Executive impairment in 31% of patients. The ALS patients demonstrated impairments in naming, comprehension and verb processing. Inspection of individual participants' performance revealed no single pattern of language impairment.

Language impairment was related to the degree of executive dysfunction; we found a significant correlation ($p < 0.001$) between language and executive composite scores. Executive functioning emerged as a significant predictor of language performance in the ALS patient group, accounting for 44% of the variance in the Language composite scores ($p < 0.001$). However, $> 40\%$ of patients classified as impaired on the language composite demonstrated executive abilities within the unimpaired range.

Discussion and conclusions: This study demonstrates that language impairments may be at least as prevalent as executive dysfunction in ALS. Results highlight that language and executive impairments are a frequent, but not consistent feature of cognitive change in ALS. Although executive and linguistic symptoms may co-occur, linguistic deficits can occur in isolation of wider cognitive change and cannot be sufficiently explained by frontal-executive dysfunction, further highlighting the heterogeneity of cognitive impairment in ALS patients.

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P207 THE BENEFIT OF USING BOSTON NAMING TEST SCORES TO CHOOSE COMMUNICATION INTERVENTION

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Keywords: communication, Boston Naming Test, augmentative and alternative communication

Background: The Boston Naming Test (BNT) measures confrontational word retrieval in individuals. A comparison

between patients in our clinical program with ALS/MND (n = 15) with a control group (n = 15) concerning subtle language disorders, showed significant differences ($p < 0.004$) between the results for the two groups on BNT (items 29–58).

Objectives: We wanted to examine if the shorter version of BNT might give us information about the lexical level of the patients and by that give us information about accurate communication intervention procedures such as different types of Augmentative and Alternative Communication (ACC).

Method: We included the shorter version of BNT in our standard clinical procedure. All ALS/MND patients joining our program at the Uppsala University Hospital, Sweden, were offered the opportunity to be examined by BNT (items 29–58; a total of 30 items). We compared the results between BNT scores among patients with already implemented ACC together with the most accurate ACC solutions for new patients. The BNT procedure was replicated no shorter than every sixth months.

Results: During March 2010 - April 2012 we examined 51 patients between one to four different occasions with the described short version of BNT. Over 90% of the intrapersonal scores were consistent (+/− 3 items). We examined 20 patients with moderate or severe dysarthria/anarthria among patients with ALS/MND with bulbar and/or higher motor neuron involvement. Three of the patients were offered ACC but denied. Two have BNT- scores < 14/30: Both use low-tech communication aids such as picture pointing or answering questions by yes/no. Six patients who scored between 16–22/30 use a mixed form of ACC with communication by a Light Writer SL35 or Speak Out, writing, pointing at pictures and letters, using a "subject chooser" or scanning by help from a significant other. Three of the six patients have whole or partially computer based communication. Nine patients scored > 22/30 and seven of these use whole or partially high-tech communication solutions such as Light Writer SL40, computer based communication through eye control or head mouse. In this high-score group there is a broader prevalence of mixed communication aids and strategies compared to the other groups. Out of the 31 patients without communication impairments we found seven patients with BNT-scores < 17/30.

Discussion: In spite of a limited group of patients we have found that BNT scores might give us an additional explanation for why some patients receive low-tech communication aids and some high-tech. By additional planning out of BNT-scores the ALS/MND patients gain more accurate communication intervention and time.

Conclusion: BNT is a helpful and easy administrated procedure that gives information which helps in choosing an accurate level of ACC on a personal basis.

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P208 A NEW TOOL TO MEASURE PATHOLOGICAL LAUGHING AND CRYING IN ALS

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Keywords: pathological laughing and crying, PBA, emotional

Background: Pathological Laughing and Crying (PLC) expresses itself as uncontrolled, unwanted emotional displays of crying and laughing and patients with Amyotrophic Lateral Sclerosis (ALS) have among the highest documented prevalence rates of PLC. The recent FDA-approved medication Neurodex has been a successful treatment option for ALS patients with PLC symptoms. Despite this advancement, our understanding of the subjective experience of PLC remains limited. The Pathological Laughing and Crying Experience Survey (PLACES) is introduced as a tool to measure patients' internal experiences of PLC, in the hope that a closer examination of the prevalence and nature of PLC will advance understanding of the anatomy of PLC, assist in identification of affected patients, and more broadly inform our understanding of emotion regulation.

Objectives: Identify the prevalence of PLC in the ALS population and validate this new tool's utility, when used with currently existing measures of PLC.

Methods: This study employed two self-administered questionnaires with ALS patients, to measure the prevalence and clinical phenomenon of PLC. The CNS-LS, a 7-item scale, identified objective levels of clinical PLC. The PLACES is an 18-item self-report likert scale that documented patients' subjective and objective experience of pathological laughing and crying episodes. Individual items measure patients' subjective experience of controllability of episodes, mood congruence, episode triggers, onset characteristics, and the duration, frequency, intensity, and type of emotional outbursts.

Results: Participants (N = 230) with confirmed ALS reported varying levels of PLC (7–33) with 35.6% reporting clinically significant levels, as indicated by a CNS-LS score of greater than or equal to 13. Of those patients with clinical levels of PLC participating in a larger study (N = 44), patients reported a preponderance of crying (52%), as opposed to laughing (24%), or equal amounts of each (24%). Male patients reported higher levels of uncontrollable laughter than did females ($F = 6.4$; $p = 0.01$), yet males and females had equal levels of crying frequency. Patients reported that both laughing and crying episodes were triggered most of the time by emotional precedence, thus they were not spontaneous. The duration of laughing episodes was shorter than crying spells, lasting only a few minutes or less, and was less congruent with pre-existing mood. Crying episodes tended to be more congruent with pre-existing mood.

Discussion: Negative emotions experienced during PLC episodes may be more authentically felt than previously suspected, being rooted in pre-existing feelings and being difficult to control. Interesting gender differences in laughing episodes warrant further study.

Conclusion: PLC in ALS patients is important to identify and treat, particularly because this common and distressing syndrome has the potential for being effectively treated.

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P209 BRAIN COMPUTER INTERFACE AND EYE-TRACKING TECHNOLOGY FOR NEUROPSYCHOLOGICAL ASSESSMENT OF ALS PATIENTS

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Keywords: cognitive assessment, brain computer interface, eye-tracking

Background: Most Amyotrophic Lateral Sclerosis (ALS) patients show a range of cognitive deficits, with 5% presenting clinical features of frontotemporal dementia (1). Cognitive assessment in moderate-severe stages of ALS may become problematic, due to the lack of motor-verbal free testing. Eye-tracking (ET) and Brain Computer Interface (BCI) have been effectively used in ALS as Alternative and Augmentative Communication means and represent interesting tools to administrate extended neuropsychological assessments.

Objectives: A recently funded project, "eBrain: BCI-ET for ALS", aims to evaluate P300 BCI and ET technology as cognitive assessment tools in ALS. To preliminary test this approach, a widespread used and sensitive cognitive task, the Verbal Fluency test (VF), has been administered to a sample of healthy subjects. The overall project testing setup has also been evaluated for successive clinical use.

Methods: Twenty-eight healthy subjects (M. age: 55 ± 14 years, M. education: 14.35 ± 3.68 years) underwent a two-hour session, including the systems calibration, a words copy task and VF with BCI and ET. In the ET assessment, the VF has been administered according to the original procedure (three one minute phonemic and semantic VF). In the BCI, subjects have been asked to produce a word starting with the "M" letter and then to write it by means of BCI, for five times; the total reasoning time has been computed. A short neuropsychological screening has also been performed.

Results: The average BCI calibration accuracy was 92.74%. In the BCI copy test, correctly selected characters were about 80% (M. 11,12 over 14); in the VF, subjects correctly written about 88% of characters (M. 24.04 over M. 27.5), with an average reasoning time of 17.5 seconds for producing five words. For the ET assessment, correctly selected characters in the copy test were about 94% (M. 30.07 over 32); in the VF, subjects correctly written about 95% of characters (M. 32.6 over M. 34.40).

Discussion and conclusions: These preliminary results show a good rate of accuracy for both BCI and ET systems, without a preliminary training. VF in BCI required not trivial changes in the original procedure, while ET allowed a reasonable adherence to the original validated test. However, BCI could be more suitable in the presence of eye movement's alterations. The definition of ALS patients' cognitive profiles has a major importance for clinical issues and patients end-of-life decisions. The study presents the use of BCI and ET as possible means for the development and administration of a motor-verbal free neuropsychological battery. This protocol will be also applied to ALS patients, by adapting other cognitive tests to these technologies in order to obtain a more comprehensive cognitive battery.

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P210 FORCED VITAL CAPACITY, NEUROPSYCHOLOGICAL IMPAIRMENT, AND AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FVC, neuropsychological, ALSFRS

Background: Forced vital capacity (FVC) is an integral and important parameter used to track and evaluate patients with MND/ALS. Previous research has linked reduced FVC with cerebral perfusion deficits in ALS. Given that decreased perfusion could result in cognitive dysfunction, there may also be a link between reduced FVC and neuropsychological deficits.

Objective: To examine the relationship between neuropsychological deficits and FVC in ALS.

Method: 32 patients diagnosed with definite and probable ALS. Patient characteristics were: 40.5% were female, 59.5% were male, M age = 62.36 (11.36); M education = 13.74 (2.35); MMSE = 27.16 (2.93); M length of illness = 4.56 (6.54); M ALS-FRS = 28.69 (11.23); and M FVC = FVC: 65.64% (23.85). Neuropsychological functioning was assessed with the Philadelphia Brief Assessment of Cognition (PBAC), brief assessment of neurocognitive functioning measuring executive control, language, visuospatial functioning, verbal/ visual memory and behavior/ comportment. The mean total PBAC score was 73.82 (10.94), a score well above the cut-off for dementia. Using simple correlation and multiple regression analyses, the combined association between FVC, neuropsychological impairment, and functioning abilities assessed with the ALS Functional Rating Scale (ALS-FRS) were examined.

Results: Simple correlations found better FVC was related to better performance on the PBAC executive Index ($r = 0.435$, $p < 0$ .004); language Index ($r = 0.428$, $p < 0.009$); visuospatial Index ($r = 0.353$, $p < 0.026$); and the total the PBAC score ($r = 0.452$, $p < 0.006$); and ALS-FRS bulbar ($r = 0.554$, $p < 0.001$; upper limb ($r = 0.427$, $p < 0.003$; lower limb ($r = 0.519$, $p < 0.519$), and respiratory ($r = 0.310$, $p < 0.038$) functioning. A regression analysis was conducted with FVC as the dependent variable. In the first block, all five PBAC neurocognitive indices were entered using a stepwise procedure. Only the PBAC executive index entered the model, accounting for 39 percent of variance ($r = 0.619$, $R^2 = 0.383$, $F = 19.21$, $df = 1/31$, $p < 0.001$). In the second block, all ALS-FRS indices were entered also using a stepwise procedure. The ALS-FRS index measuring lower limb functioning entered first ($r = 0.770$, $R^2 = 0.593$, $F = 15.48$, $df = 1/30$, $p < 0.001$), followed by ALS-FRS bulbar index ($r = 0.770$, $R^2 = 0.666$, $F = 6.30$, $df = 1/29$, $p < 0.018$). The entire model accounted for 66 percent of the variance.

Discussion: In prior research ALS severity, operationally defined with the ALS-FRS and FVC, correlated with reduced cerebral perfusion in frontal/ parietal brain regions assess with arterial spin labeling (ASL). The current research found an association between executive function and ALS-FRS lower limb followed by bulbar functioning suggests that FVC might be used to estimate cerebral functioning in ALS patients.

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P211 DIFFERENTIAL NEUROPSYCHOLOGICAL IMPAIRMENT ASSOCIATED WITH BULBAR ONSET AND INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: executive dysfunction, bulbar disease

Background: ALS patients with bulbar onset typically have a poor prognosis though prolonged survival may occur. Dementia and executive dysfunction are additional poor prognostic factors in ALS. There remains controversy as to whether bulbar onset disease is associated with an increased incidence of executive impairment.

Objectives: To test the hypothesis that bulbar involvement in ALS patients will be related to greater neuropsychological impairment.

Methods: A group of approximately 200 patients were seen at the ALS Association Center at the University of Pennsylvania and participated in ongoing longitudinal research (M age = 61.13 + 13.05; M education = 14.31 + 3.40; M MMSE = 28.45 + 2.53). Data regarding 1) the site of disease onset; and 2) the presence or absence of upper motor neuron (UMN) and lower motor neuron (LMN) disease by segment was collected. Neuropsychological assessment included a letter F-fluency test, digits forward/ backward, The Oral Trails Test, and a modified Visual-Verbal Test; not all tests were administered to all patients. The effect of initial segment involvement at disease onset (bulbar, cervical, lumbosacral) was assessed with a series of 1-way ANOVAs.

Results: When compared to patients with cervical or lumbosacral onset, patients with bulbar onset disease presented with lower performance on the digits backward and Visual-Verbal tests (digits backwards $F(2/154) = 4.17$, $p < 0.017$; Visual-Verbal Test ($F(2/97) = 3.82$, $p < 0.025$). Post-hoc analyses found that bulbar onset disease patients repeated fewer digits backward compared to patients with cervical onset disease ($p < 0.012$) and scored lower on the Visual-Verbal Test compared to cervical onset ($p < 0.032$) and lumbosacral onset ($p < 0.048$) patients. Groups were then constructed on the basis of presence or absence of UMN and/or LMN signs for all three segments. T-tests comparing patients with UMN bulbar disease versus no UMN bulbar disease found worse performance on tests of letter F-fluency ($t(184) = 2.38$, $p < 0.018$), digits backwards ($t(174) = 2.05$, 0.042) and Oral Trails ($t(157) = 2.84$, $p < 0.005$). Similar t-test compared for the presence or absence of UMN and LMN cervical and lumbosacral signs revealed no differences on neuropsychological tests.

Discussion: In a large cohort of ALS patients, bulbar onset disease and bulbar involvement, and particularly UMN bulbar involvement, appears to result in differential neuropsychological impairment when compared to other groups of ALS patients.

Conclusions: Longitudinal research, currently underway, will address whether bulbar onset and the presence of UMN bulbar signs are differentially related to the cognitive impairment throughout the disease course.

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P212 GENDER DIFFERENCES IN THE BEHAVIORAL VARIANT OF FRONTOTEMPORAL DISEASE (BVFTD) IN AMYLOTROPHIC LATERAL SCLEROSIS: A NATIONAL SAMPLE

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Keywords: FTD, gender, remediation

Background: We recently demonstrated gender differences in emergence of frontotemporal disease (FTD) associated cognitive decline in a national sample of 110 ALS subjects(1). Female gender was associated with more bilateral involvement of cognitive change, with relatively greater preservation of language capacities, consistent with cognitive reserve theory. Currently, we hypothesize that female gender is associated with a less florid, more apathetic profile in ALS in the course of emerging behavioral change, consistent with more frontal cortical-subcortical involvement.

Objectives: Our objectives were to investigate gender differences in prevalence rates and pattern of emergence of change in the three recognized subtypes of behavioral impairment (bi) in ALS: disinhibited (DIS), apathetic (APA) and stereotypic (STE)(2).

Methods and materials: A convenience sample of 114 subjects (56 M) from 14 ALS multidisciplinary clinics were evaluated cross-sectionally by completion of the 24 item Frontal Behavioral Inventory (FBI), a caregiver based interview rating of behavioral change with scores ranging from 0–3. Based upon Neary *et al.* classification(2), 5 FBI items were combined for each behavioral subtype. This allowed for a range of scores from 0–15, while data for each gender was skewed due to the mild degree of behavioral change evidenced by most subjects. Thus, the non-parametric Mann-Whitney U was applied to compare gender group findings. Demographic comparisons were made with t-tests.

Results: Gender groups were equivalent for education and IQ, the female group expectedly older($p = 0.006$). Prevalence rates of clinically evident behavioral change (FBI Total > 27) were small for the total sample (4.1%) and equivalent between gender groups ($p = 0.234$). No gender differences were evident for the APA and STE subtypes, while a significantly greater proportion of males evidenced the DIS subtype($p = 0.002$).

Discussion and conclusions: Consistent with the tendency to mask cognitive change in emerging FTD(1), female

gender appears to be associated with a more covert type of behavioral change in the early stage. Ironically, this is recognized to be associated with more widespread frontal pathology extending into the dorsolateral convexities of the frontal lobes(2). We propose a remediation approach based upon a neuroendocrine model purporting the use of estrogen as an effective intervention strategy to impede both the declines in cognitive capacities and behavioral activation evidenced in female ALS patients with emergence and progression of FTD. We plan to test this neuroendocrine model in a multicenter clinical trial involving FTD patients in the presence and absence of ALS, in a crossover design.

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P213 NARRATIVES OF ALS: A GENDERED MODEL

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Keywords: narratives, illness experience, gender

Background: Narratives of illness, disability, mental disorders, genetic disorders, chronic conditions, dying and death play a key role in shaping our understanding of the human condition. In the past decades efforts have been made to better understand these anthropological illness narratives, with an increasing interest on chronic illness. This research project is a cross-sectional anthropological study of the illness experience of men and women with ALS. ALS presents a particular set of challenges for the construction of an illness narrative, since it has no known etiology, no treatment, and no cure. A critical medical anthropological approach is used to examine the limits and challenges of biomedicine through the lived experience of men and women with a visible and life-limiting chronic illness.

Objectives: The research objectives were: 1) determine how health behaviors (with regard to terminal illness) vary between genders 2) identify the impact of gender on the attitudes of men and women with ALS related to family, emotional support, identity, and explanatory models 3) understand how individuals cope with an illness such as ALS with no known etiology, no treatment and no cure.

Methods: The study used nine participants, five men and four women living in Georgia. Qualitative and quantitative methods were used to collect data, including: semi-structured interviews, structured questionnaire including psychosocial instruments to assess coping style, and participant observation of support group meetings

Results: Results identified both gendered and universal themes among individuals with ALS, and reflect various coping measures and health behaviors that emerge for men and women with ALS.

Discussion: The narratives of female persons with ALS showed the following themes: a longing for aesthetic beauty/feminine qualities, maternal concern/compassion, spousal anxiety, and acceptance. Male ALS narratives uniquely dealt with an acute awareness of diagnosis date, lack of familial provision/protection, dependability, weight and body image, sexual performance and physical activity. Illness narratives provide clinicians and researchers insight about ALS and improve quality of life for patients and their families.

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P214 KINSHIP DIFFERENCES IN CAREGIVER BURDEN

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Keywords: caregiver burden index, caregiver burden, kinship

Background: Caregiver burden in Amyotrophic Lateral Sclerosis (ALS) is arousing an increasing attention, since the psychosocial demands posed by such a disease are remarkable. In this regard, further investigation is needed to identify differences in burden among specific caregiver subgroups. In our study we evaluated caregiver burden in relation to kinship, with the aim of verifying if different relationships and roles within the family might be related to different burden perceptions.

Patients and methods: We have administered the Caregiver Burden Inventory (CBI) to 72 caregivers (mean age ± SD: 53.4 ± 11.5 yrs; 24 M; 48 F) of ALS patients, and differences related to sex and kinship have been explored comparing subgroups by t-test.

Results: Scores of physical and social burden are significantly higher ($p < 0.05$) in the general female subgroup. Partners show higher rates ($p < 0.05$) of physical burden and higher total scores ($p < 0.01$) than sons and daughters. Among adult children caregivers, daughters complain higher developmental ($p < 0.05$) and general burden ($p < 0.05$) than sons.

Discussion: Data analysis suggests significant differences in caregiver burden according to sex/gender and type of relationship with the patient. The most representative subgroup of caregivers is that of partners (especially wives): in such a subgroup caregiver burden seems to be higher. Moreover, differences within the general sample, as well as in the subgroups, support the concept of a higher burden in females.

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THEME 10 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

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THEME 10 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P215 ASSESSING COGNITIVE CHANGES IN ALS: FROM A NOVEL BEHAVIORAL TEST TO ITS NEURAL CORRELATES

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Keywords: cognitive testing, MRI, frontal lobe function

Background: ALS is viewed as a multisystem disorder (1), yet its cognitive changes are difficult to identify in the clinic largely due to the relatively mild nature of the deficits, limitations of the standardized neuropsychological assessments in patients with severe motor impairments, and the lack of agreement regarding the nature and origin of the cognitive decline.

Objectives: The goals of this study are: A) To assess sensitivity of a novel Computerized Frontal Battery (ALS-CFB), which links cognitive processes to specific brain regions (2), against a standard neuropsychological battery; and B) To measure the association between cognitive impairment and structural changes in the brain.

Methods: Eighteen patients with ALS and the same number of matched healthy controls were recruited. Ten patients with ALS returned for a repeat assessment in approximately 8 months. Structural MR included T1W (3D) and interleaved 2D dual TSE PD/T2. A novel Semi-Automated Brain Region Extraction (SABRE) approach (3) was used to perform a volumetric analysis in the gray (GM) and white (WM) matter as well as sulcal and ventricular CSF and subcortical hyperintensities of 30 segmented regions. Cognitive testing included ALS-CFB, composed of tests sensitive to frontal processes such as energization (i.e., ability to initiate and sustain a response), executive functioning, emotion regulation and metacognitive processing, as well as the standardized neuropsychological battery.

Results: ALS-CFB detected changes in cognition when the standardized testing failed to do so. Significant atrophy was observed in the GM of the inferior frontal (IF) and anterior temporal (AT) regions as compared to the controls ($p < 0.05$). Longitudinally, the atrophy was seen in the GM in the medial inferior frontal cortex bilaterally and in the WM in the middle frontal (MF), inferior frontal (IF) and medial middle frontal (MMF) regions on the right. Sulcal CSF increased in the same regions. Significant correlations were observed between GM atrophy in the IF region and reaction time and anti-saccades testing and AT atrophy correlated with digit forward task across participants. Longitudinally, IF and MF atrophy was correlated with changes in verbal fluency and reaction time.

Discussion and conclusions: ALS-CFB detected impairments in energization, motor inhibition as measured by saccades, and executive function in the ALS group. These changes were missed by the standard neuropsychological battery. Additionally, MR imaging revealed volumetric changes in the GM, WM and sulcal CSF predicted by the frontal lobe model loci (IF, MF) (2). The results demonstrate the sensitivity and utility of the ALS-CFB in detecting region specific frontal lobe dysfunction, which is correlated to structural changes on neuroimaging.

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P216 FRONTOTEMPORAL PATTERN OF NEURODEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS: A CORTICAL THICKNESS 3T MRI STUDY

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Keywords: MRI, cortical thickness, frontotemporal lobar degeneration (FTLD)

Background: The extensive application of advanced magnetic resonance imaging (MRI) techniques has undoubtedly improved our knowledge of ALS pathophysiology. Nevertheless, the actual spread of the neurodegenerative process throughout the central nervous system is not fully understood.

Objectives: The aim of the present study was to assess the spatial distribution of cortical damage in ALS, by using a cortical thickness (Cth) measurement approach.

Methods: Automatic surface-based Cth measurements were performed on structural 3T MRI data of 20 ALS patients and 18 matched healthy controls (HC) in a case-control study design. Clinical scores of disability and disease progression were correlated with Cth measures.

Results: Comparing patients with controls, we observed significant cortical thinning mainly in frontotemporal areas,

bilaterally, including the primary motor, lateral frontal and prefrontal, medial frontal, temporal and parieto-occipital cortices. Furthermore, cortical thinning was significantly related to i) ALS Functional Rating Scale-Revised (ALSFRS-R) score (an index of disease disability) in a left lateral frontal area ($p = 0.0356$), ii), disease progression rate in a left medial temporal area ($p = 0.0265$), and iii) to disease duration in a right medial frontal area ($p = 0.0072$).

Discussion and conclusions: Cortical thinning of the primary motor cortex might be a diagnostic marker for upper motor neuron degeneration in ALS. The correlations found between cortical thinning of frontotemporal areas and clinical measures of disability and disease progression as well as disease duration might provide a further evidence of the progressive development in ALS of a multisystem disorder within the spectrum of frontotemporal lobar degeneration (FTLD).

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P217 CORTICAL THICKNESS IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTO-TEMPORAL DEMENTIA

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Keywords: *cortical thickness, cognitive impairment, ALS-FTD*

Background: A continuum between amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) is encouraged by pathological, biochemical and genetic characteristics. Overlapping cognitive and behavioural profiles in patients with ALS and FTD have been demonstrated by neuropsychological studies. Imaging studies showed that FTD patients have a distinct pattern of cortical thinning in the temporal lobe which is furthermore associated with progression of clinical deficits (1).

Objectives: Our objective was to compare the cortical thickness of ALS patients with different cognitive stages ranging from normal cognitive performance up to dementia (ALS-FTD). We expected a similar pattern of cortical thinning wherein the ALS patients with no cognitive impairment (ALSnci) should have a stronger thinning in motor areas and ALS-FTD patients in frontal and temporal regions. The patients with cognitive impairment (ALSci) should show a pattern involving motor, frontal and temporal regions.

Methods: An age and gender matched sample of 69 ALS patients and 34 healthy controls was investigated. The ALS group was divided by their cognitive status and according to current consensus criteria (2, 3): 33 ALSnic, 28 ALSci, 8 patients were diagnosed as ALS-FTD. The cortical thickness was measured using FreeSurfer. A vertex-wise comparison was performed using a GLM and results are reported according to the Desikan-Killiany atlas.

Results: A vertex-wise comparison between all four groups revealed the following significant results (for all $p < 0.05$, FDR corrected): ALS-FTD patients had a thinner cortex as the ALSci who had a thinner cortex as the ALSnci patients (ALS-FTD < ALSci < ALSnci) in left and right superior temporal gyrus, superior frontal gyrus, left caudal middle frontal gyrus, lateral orbital frontal gyrus (for ALSnci > ALSci both hemispheres). Besides, a thinner cortex of ALSci patients compared to ALSnci patients was found in the left pars opercularis and rostral middle frontal gyrus. Additionally, a thinner cortex of ALS-FTD patients compared to ALSci patients could be demonstrated in the posterior cingulate cortex. Only the comparison between healthy controls and ALSci patients revealed significant results in terms of significant thinning in the left and right precentral gyrus, pars opercularis, insula, superior and middle temporal gyrus, posterior cingulated cortex and left middle and superior frontal gyrus and medial orbital frontal cortex.

Conclusion: As expected, alterations in the brain of patients with cognitive impairment could be found in the temporal and frontal regions. The decreasing thickness of the fronto-temporal cortex from ALSnci via ALSci to ALS-FTD appears to be an indicator for the continuum between ALS and FTD. Further research is needed to explore cortical thickness as prognostic marker.

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P218 DIFFERENTIAL INVOLVEMENT OF CORTICOSPINAL TRACT FIBERS IN UMN-PREDOMINANT ALS PATIENTS: A DIFFUSION TENSOR IMAGING AND TRACTOGRAPHY STUDY

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Keywords: tractography, hyperintensity, truncation

Background: Diagnosis of amyotrophic lateral sclerosis (ALS) is dependent on clinical evidence of combined upper motor neuron (UMN) and lower motor neuron degeneration, although either can predominate at disease onset. Some UMN-predominant ALS patients display bilateral hyperintensity of the corticospinal tract (CST) on T2- and proton density (PD)-weighted MRI sequences. Interestingly, other UMN-predominant ALS patients, phenotypically indistinguishable, do not have such CST hyperintensity. The reason for this variability between presence or absence of CST hyperintensity and the essentially identical UMN-predominant clinical features is unclear.

Objectives: Using diffusion tensor imaging (DTI) with diffusion tensor tractography (DTT) to quantitatively assess the intracranial CST of UMN-predominant ALS patients with or without hyperintensity could possibly reveal differences in DTI abnormalities along the CST of both patient subgroups.

Methods: DTI data were obtained at 1.5T in 47 UMN-predominant ALS patients with ($n = 21$) CST

(ALS-CST +) or without ($n = 26$) CST hyperintensity (ALS-CST-) and in neurologic controls ($n = 12$). Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were compared between patients and controls along the CST. ALSFRS-R score, disease duration, disease progression rate, and El Escorial score were also obtained in ALS patients.

Results: Significant abnormalities ($p < 0.05$) were detected in FA, AD, or RD in CST primarily at the internal capsule (IC) level in ALS patients, especially in the ALS-CST + subgroup. AD and RD values were different between ALS-CST + and ALS-CST- patients suggesting distinct pathologies between these subgroups. Furthermore, DTT revealed subcortical truncation of fibers projecting from the precentral gyrus in some ALS-CST + and ALS-CST- patient subgroups but in no controls; fibers projecting to the adjacent postcentral gyrus were spared. This suggests an abnormality of CST fibers arising from primary motor cortex of UMN-predominant ALS patients and not of fibers projecting to/from the primary sensory cortex. Significantly shorter disease duration ($p = 0.02$) and faster disease progression rate ($p = 0.03$) were observed in ALS patients with CST fiber truncation than those without, whereas no significant differences were noted for ALSFRS-R and El Escorial scores.

Discussion and conclusions: Abnormalities in DTI metrics at the IC level suggest axonopathy in UMN-predominant ALS patients, which can be quantitatively distinguished between those with CST hyperintensity and those without. Our DTT finding of subcortical fiber truncation in patients arising from the primary motor cortex and not those projecting to/from the primary sensory cortex suggests specificity of this finding, and is in keeping with relative sparing of the sensory system in ALS. Correlation of CST fiber truncation with shorter disease duration and faster disease progression rate in these UMN-predominant ALS patients suggests that DTI/DTT can identify a more severe pathologic process in some patients and be used to distinguish between patient subgroups.

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P219 THE RESTING STATE DEFAULT MODE NETWORK (DMN) IS PATHOLOGICALLY HYPERACTIVE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: resting state network, default mode network, dual regression

Background: Resting state networks are activated in the human brain when the subjects is at rest (closed eyes, non sleeping and asked to think about nothing). They can be assessed using BOLD effect based functional MRI-techniques. The default mode network (DMN) is active in the medial and lateral part of the prefrontal and temporolateral cortex. The DMN intensifies its activity at rest; its function is thought to be related to daydreaming, comprehending emotions of other people, building autobiographic memory and others functions. In ALS, the DMN has been shown to be activated in larger areas than normal in sensorimotor regions, but premotor and frontal involvement was not decisive.

Objective: To determine the extent of the DMN in ALS patients MRI where T1 spatial normalization was optimized to reduce misrepresentation artefacts.

Methods: We compared 40 Patients with ALS against 40 age matched healthy controls, using the FSL Software package (http://www.fmrib.ox.ac.uk/fsl/index.html). We performed detailed spatial normalization and skull stripping using SPM and an independent component analysis using the FSL Dual Regression Tool (http: //www.fmrib.ox.ac.uk/analysis/dualreg) for the comparison of the network activity on group level. All Subjects underwent an assessment of clinical questionnaires (ALSFRS, SF36, EuroQual5D, Frontal Assessment Battery, Mini-Mental Status Examination and Edinburgh Handedness Inventory).

Results: We found a distinctly different behaviour of the DMN of ALS patients when compared to controls. In particular the DMN in ALS significantly increased in both the frontal and temporal regions of the DMN highly suggestive of a loss of intracortical inhibition.

Discussion: These findings may represent compensation for lost underlying subcortical neuronal network capacities or indeed the loss of anatomically long tract connectivity itself when intrinsic intracortical inhibition is postulated to be dependent on anatomically intact long tract connectivity (as opposed to U-fibre connectivity). Previously, a similar analysis (1) was performed, but they found much less hyperactivity in fewer regions of the DMN in patients. Using adequate preprocessing (i.e., optimized skull stripping) greatly improves detection of the extent of DMN hyper- and hypoactivity, and thus the value of rsfMRI to describe ALS related alterations of brain connectivity.

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P220 VOXEL-BASED INTENSITOMETRY OF T1 MRI CAN DIAGNOSE ALS AND PREDICTS CLINICAL DETERIORATION

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Keywords: MRI, biomarker, diagnostic tool

Background: We introduce a novel approach to advanced voxel-based MRI analysis called Voxel-based intensitometry (VBI). Utilizing known approaches like VBM we developed VBI as a technique to reveal and assess white matter damage in ALS, which is more consistent and more easily accessible than grey matter alteration.

Objectives: To submit T1 MRI data of 30 patients (mean age 63, SD 11; mean ALSFRS-R 37, SD 6; mean disease duration 24 months, SD 18) and 37 matched healthy volunteers to in-house developed preprocessing algorithms that allow for direct comparability of intensity information and assess its feasibility for diagnosing cerebral involvement in motor neuron disease.

Methods: Patients and controls were subjected to T1 MRI and comprehensive clinical assessment, including ALSFRS-R. VBM-like preprocessing algorithms were adapted to normalize all datasets in space and average intensity. Group comparison ANCOVA was used to identify significant ALS-related clusters of altered T1 signal, in which mean intensity was calculated. Regression analysis was conducted to find regions where intensity and ALSFRS-R scores correlated.

Results: Group comparisons revealed areas of significantly different intensity between patients and controls. These regions comprehensively overlapped with the known changes in the white matter of ALS patients' brains, such as pyramidal tract, corpus callosum, frontal regions and known regions of extra motor involvement. In these clusters, mean intensity allowed to separate patients and controls with 89% specificity at 100% sensitivity. Furthermore regression analysis identified ROIs in which averaged intensity significantly correlated with ALSFRS-R scores (Spearmans rho = −0.89**).

Discussion: In our cohort VBI identified patients vs. controls. Additionally the correlation between intensity and clinical scores suggests predictability of clinical status of the patients on behalf of their MR scans. If proven true, VBI would be the first objective tool to diagnose ALS and verify clinical status in addition to clinical assessment and ALSFRS-R. These results have to be verified in larger cohort multi center trials to prove VBI as a competent biomarker.

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P221 A LONGITUDINAL FMRI STUDY OF THE ACTIVATION PATTERN IN MOTOR AND EXTRA-MOTOR AREAS IN ALS

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Keywords: fMRI, hippocampus, biomarker

Background: Amyotrophic lateral sclerosis (ALS) is increasingly recognized to be a multisystem disease associated with cognitive dysfunction. Conversely, functional neuroimaging studies revealed activation changes in motor-related areas, but sometimes also within fronto-temporal regions. Typically, activity elicited by a certain task is compared in ALS vs. healthy controls. However, prospective studies investigating the functional changes related to the disease-progression of ALS in a longitudinal within-subject design are lacking.

Objectives: In the present study, we investigated the time course of changes in fMRI activation patterns and their potential contribution to the understanding of ALS pathophysiology.

Methods: Motor- and novelty-processing related brain activity was analyzed in patients with ALS (n = 14) in two sessions separated by a 3-month interval. To assess motor-related activity we employed a Go/NoGo-task, in which task-irrelevant novel stimuli (inside and outside scenes) were additionally presented, offering the possibility to investigate the neural processing of stimulus novelty relative to frequently presented stimuli.

Results: The comparison of the results of the first and second measurement revealed that the activity in the motor cortex decreased during the time course of 3 months. This might reflect the progressive major neural loss in the motor system and functionally connected areas. Interestingly, the activation-decrease in the cerebellum correlated positively with the patients' ALSFRS-Score and in motor cortex with their MRC-Megascore. Importantly, novelty-processing related activity in the hippocampus was higher in the second compared to the first measurement. This activation-increase showed a negative correlation with the patients' ALSFRS-Score. This pattern most likely reflects compensatory activity in order to overcome dysfunctions typically observed at the beginning of lesions.

Conclusions: This longitudinal fMRI study captures progression of motor and extra-motor degeneration in ALS. The functional pattern points clearly to an involvement of the hippocampus. Compared to the motor system lesion, the hippocampal dysfunction occurs at a later disease stage which might explain why memory impairment is hardly detected in a clinical setting.

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P222 PATIENTS WITH ALS SHOW ALTERED ADIPOSE TISSUE DISTRIBUTION IN WHOLE BODY MRI ANALYSIS

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Keywords: adipose tissue, MRI, automated analysis

Introduction: Since neurodegenerative diseases affect body weight, assessment of the body fat distribution in the course of the disease might act as a surrogate marker. Body mass index and blood lipids have already been reported to correlate with survival and functional status of ALS patients (1, 2). However, these techniques provide no information on the regional distribution of adipose tissue in ALS patients but there are important metabolic differences between fat depots. In this study, whole body MRI was used to analyze fat tissue volume differences between ALS patients and controls.

Methods: Whole body MRI data were acquired on a 1.5 T scanner by acquisition of 6 to 8 consecutive T1-weighted volumes. Sixty-two patients with definite or probable ALS according to revised El Escorial criteria (age 60 ± 12 years, ALSFRS 36.3 ± 7.5, mean disease duration 22 months) and 62 age- and gender-matched controls were examined by a standardized automatic image postprocessing protocol (3), subcutaneous and visceral fat volumes were determined by the Automatic Tissue Labelling Analysis algorithm. Data were diffusion filtered prior to application of the fat determination algorithm ARTIS (Adapted Rendering for Tissue Intensity Segmentation).

Results: The region-of-interest-based approach could be restricted to parts of the body where major fat volume changes were expected in the course of the disease so that MRI-based fat volume analysis was able to differentiate between visceral and subcutaneous fat tissue. Total fat volume of ALS patients was not changed as compared with controls. ALS patients displayed decreased

and increased visceral fat content and thus an increased ratio of visceral to subcutaneous fat. Multiple regression analysis showed that gender and ALS-FRS-R were significant predictors of fat volumes.

Conclusion: The potential in determination of subcutaneous and visceral fat volume by MRI based analysis was demonstrated. Fat distribution was altered in ALS patients with increased visceral fat compared to controls. These findings demonstrated that adipose tissue was affected in its topography in ALS and calls for further functional studies on this key metabolic tissue.

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P223 A LONGITUDINAL STUDY OF DIFFUSION TENSOR IMAGING IN ALS PATIENTS

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Keywords: DTI, fractional anisotropy, corticospinal tracts

Background: DTI measures the direction of the flow of water along axon tracts. Fractional anisotropy (FA) derived from DTI serves as an *in vivo* marker of the integrity of these tracts. Degeneration of these tracts will result in a reduced FA value.

Objective: To assess changes in white matter tracts integrity in ALS patients compared to controls, early in the disease and at 6 months.

Methods: 12 patients with probable and definite ALS with FVC > 70% and ALSFRS > 35 and 12 healthy controls were enrolled. Subjects were scanned at baseline and 6 months later. ALSFRS-R, FVC and manual muscle testing were performed at baseline and at 6 months. Patients and controls underwent two structural MRI scans at baseline and at 6 months that included a high resolution T1 and a DTI scan. We calculated voxel wise fractional anisotropy (FA) Voxel-wise statistical analysis of FA data was carried out using the tract-based spatial statistics. A mean FA skeleton was created for each group. Also, the T1-anatomical brain images were used to calculate cortical gray matter volume.

Results: All ALS patients exhibited a significant progression in their disease over a period of 6 months. When comparing the whole brain skeletal FA between ALS patients and controls we found that the region corresponding to the bilateral corticospinal tracts caudal to the thalamus had significantly lower FA in ALS patients. Over 6 months the anisotropy for this region did not exhibit a significant change, but showed a decreasing trend. This decrease in FA correlated with the decline in FVC. The anisotropy of part of the superior longitudinal fasciculus that was not affected at baseline seemed to decrease in ALS patients but not in controls over 6 months. For the area where ALS patients showed a significant difference from control baseline, there was a difference in radial but not in axial diffusivity suggesting that disrupted myelin tracts may be at the root of the drop in FA noted in the patients. The correlation between peripheral grey matter and mean skeletal FA observed in the healthy controls was disrupted in ALS patients.

Discussion and conclusion: The significant decrease in FA values noted in ALS patients very early in the disease process suggest that DTI is a sensitive tool for detecting early corticospinal tract degeneration and may help establish early diagnosis. Surprisingly longitudinal analysis did not reveal significant change in FA values overtime. Longitudinal decline in FA has been found by some investigators but not by others. DTI could be used as a surrogate marker of UMN degeneration but further studies need to be done before using it as a marker of disease progression. We also found that our patients had difficulties tolerating repeated MRI scan as their disease progressed.

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P224 CORTICAL HYPEREXCITABILITY PRECEDES THE CLINICAL ONSET OF SPORADIC ALS

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Keywords: cortical hyperexcitability, precedes clinical onset, sporadic ALS

Objective: The pathophysiological mechanisms underlying the development of sporadic amyotrophic lateral sclerosis (ALS) remains unresolved. Cortical hyperexcitability was noted to precede the clinical onset of familial ALS in previous studies. This issue remains a matter of debate in sporadic ALS and was addressed in the present study which assessed cortical excitability from a clinically and electro-physiologically normal region in early sporadic ALS patients.

Methods: Cortical excitability studies were undertaken in 12 sporadic, Awaji Criteria probable, ALS patients (6 Male; 6 Female, mean age 56 years) using the threshold tracking technique of Transcranial Magnetic Stimulation (TMS). Motor Evoked Potentials (MEP) were recorded from a clinically normal abductor pollicis brevis (APB) muscle which was also assessed by qualitative and quantitative electromyography (EMG). Results were compared to 66 age matched healthy controls.

Results: Short-interval intracortical inhibition (SICI) was significantly reduced in ALS patients when compared to controls (averaged SICI ALS $0.3 \pm 0.8\%$; controls $10.5 \pm 0.7\%$, $P < 0.0001$) along with a reduction in the resting motor thresholds ($P < 0.05$) and cortical silent period ($P < 0.05$) and an increase in intracortical facilitation ($P < 0.05$), all indicative of cortical hyperexcitability. Importantly, there was no neuro-physiological evidence of LMN dysfunction, within the target APB muscle. Motor unit architecture represented by the mean motor unit potential (MUP) amplitude, duration and polyphasia was normal

Conclusion: The present study confirms that cortical hyperexcitability precedes the onset of lower motor neuron dysfunction in sporadic ALS, thereby suggesting a central origin of sporadic ALS with cortical hyperexcitability underlying motor neuron degeneration.

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P225 SERIAL MOTOR UNIT NUMBER INDEXING (MUNIX) STUDIES IN THE BICEPS MUSCLE OF ALS PATIENTS

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Keywords: motor unit, motor unit number estimation, motor unit number index

Background: Motor unit number estimation (MUNE) studies have been used to study loss of motor units (MUs) in patients with motor neuron disease (MND). MUNE is usually performed in distal muscles of hand, e.g., thenar or hypothenar. Proximal muscles are difficult to study due to the multiple nerve stimuli required. A new method called 'motor unit number index (MUNIX)' requires only a single supramaximal stimulation with optimized surface electrode placement, and uses surface EMG interference pattern (SIP) for analysis. Hence it can be used to study large proximal muscles.

Objective: To investigate the change in the number and size of MUs in biceps using the MUNIX method.

Methods: Studies were performed in fourteen patients with MND at 3 month intervals. All patients had 5–7 such studies over a 12–18 month period. The compound muscle action potential (CMAP) was recorded using supramaximal intensity. Several active electrode positions were tested to record the response with highest amplitude. The SIP was recorded using isometric contraction at force levels ranging from slight to maximum. MUNIX was computed using the area and power of the CMAP and SIP signals (1). Motor unit size index (MUSIX) was computed by dividing CMAP amplitude by MUNIX.

Results: Patients were divided into 2 groups based on the disease onset in upper limb (group 1) or lower limb/bulbar (group 2). In Group 1 (5 patients), the MUNIX decreased by 54% while CMAP decreased by 42%. MUSIX increased by 40% over the study period. Three patients had reduced CMAP and MUNIX with increased MUSIX. In group 2 (9 patients) the CMAP and MUNIX decreased by 17% and 18%, respectively, and MUSIX increased by only 2%. One patient had reduced CMAP and MUNIX and with increased MUSIX. Another two patients had borderline abnormalities.

Discussion: This study demonstrates greater MU loss in the biceps muscle of patients whose disease onset was in the upper limb. The reduced MUNIX was partially compensated by reinnervation which gave increased MUSIX with a smaller drop in the CMAP amplitude. In the second patient group, the biceps showed fewer changes in CMAP, MUNIX and MUSIX. Most patients had normal findings in each of 5–7 investigations. This reflects less involvement and progression of the biceps. MUNIX was easy to perform, requiring less than 5 minutes. Recording the CMAP with maximal amplitude was easier when the response from the previous study is available for comparison.

Conclusion: MUNIX can be used to study MU loss and remodeling in MND in proximal muscle such as the biceps.

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P226 BIOELECTRICAL ACTIVITY IN MUSCLES OF PATIENTS WITH MOTOR NEURON DISEASE: QUANTITATIVE MUP ANALYSIS, REPETITIVE NERVE STIMULATION AND SINGLE FIBER EMG

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Keywords: Single fiber EMG, repetitive nerve stimulation, quantitative motor unit potential analysis

Background: Electrodiagnostic examination reveals the findings of lower motor neuron (LMN) involvement in ALS which is characterized by ongoing denervation and reinnervation. The rate of disease progression and outcome depend on the dominant process either denervation or reinnervation. As well as the typical routine EMG findings of ALS, the tests which evaluate neuromuscular junction (NMJ) might be pathologic reflecting the crippled transmission caused by immature axonal sprouts and end-plates.

Objective: In this study, bioelectrical activity of motor units in MND was evaluated by Q-MUPA, RNS, SFEMG.

Methods: Thirty two patients (13 F, 19 M; 19–74 years old) who had been diagnosed as MND by clinical and electrophysiological findings were enrolled. Repetitive nerve stimulation of ulnar and accessory nerves as well as Q-MUPA and SFEMG in m. EDC had been performed.

Results and discussion: Mean disease duration was 36.9 ± 60.3 months. In Q-MUPA, MUP durations were prolonged in 28 patients and 7 of them had also high amplitude values. In RNS, 18 patients showed significant decrement (13 patients on m.TRP, 3 patients on m.ADM, 2 patients on both m.ADM and m.TRP). However, 13 of the patients didn't have a significant decrement. SFEMG revealed high jitter values in 29 patients but 2 patients with normal strength in their EDC muscles revealed normal jitter. In 1 patient, both RNS and SFEMG could not be performed.

Conclusion: These findings demonstrate an ongoing reinnervation in MND by means of long duration - MUPs and faulty transmission at NMJ caused by collateral sprouting. The MUP changes and NMJ abnormality could be detected by Q-MUPA and RNS and/or SFEMG, respectively.

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P227 EEG SOURCE ANALYSIS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: qEEGt, source analysis, brain electrical tomography

Background: Traditional electroencephalography (EEG) analysis has been considered of scant importance in Amyotrophic Lateral Sclerosis (ALS) because it does not show any particular slowing or specific abnormal activity in this disease, nevertheless the few quantitative EEG(QEEG) studies carried out in ALS has shown evidences of subtle EEG changes in alpha frequencies. No EEG sources analysis studies have been performed in ALS patients.

Objectives: This work focuses on the application of quantitative electric tomography (qEEGT) analysis for determine and localize cortical sources of subtle, not visually detected, changes in EEG activity (for all frequency bands) in ALS patients without dementia.

Methods: conventional basal EEG (19 electrodes, 10/20 system) were recorded from 15 ALS patients in a relaxed wakefulness stage with eyes closed. Bayesian model averaging (BMA) approach was used to estimate electrical sources of EEG recorded activity. High resolution source Z-spectra and 3-dimensional images (Brain Electrical Tomography) of Z values for all the sources at each frequency (0.5-30 Hz) were obtained for all cases. To estimate statistically significant increments and decrements of brain electric activity within the frequency spectra, the t-Student vs. Zero test was performed.

Results: Evident changes were found at the level of EEG cortical sources in all ALS patients independent of time disease evolution. Significant decrement of alpha activity was found at the pre and potcentral gyrus, and in the occipital cortex, cuneus and precuneus areas of both hemispheres. Significant increment of theta activity was found in the precentral and postcentral gyrus, supplemented motor area, superior and middle frontal gyrus of both hemispheres.

Conclusions: This study documents the presence of widespread cortical dysfunction suggesting pathological involvement beyond the motor areas in ALS patients, including those with recent onset of their disease. qEEGt analysis is likely to be a powerful tool for detecting and localize subtle EEG changes in ALS patients.

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P228 THE CLINICAL AND NEUROPHYSIOLOGIC STUDY OF UPPER MOTOR NEURON-DOMINANT AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: PLS, upper motor neuron-dominant ALS, characteristics

Objective: To investigate the clinical and electrophysiological characteristics of upper motor neuron-dominant amyotrophic lateral sclerosis (UMN-D ALS).

Methods: The clinical and electrophysiological characteristics were analyzed retrospectively in 76 patients with UMN-D ALS and 19 patients with primary lateral sclerosis (PLS).

Results: There were 84 UMN-D ALS patients after 8 PLS patients shifted to this group. In UMN-D ALS patients, the females increased in those with ages more than 40 (male: female = 1: 1.37), 32 patients (38.1%) were bulbar onset. UMN-D ALS patients acquired EMG evidence of denervation in a median of 30 months after symptom onset, and clinical lower motor neuron (LMN) signs 6 months later, 77 patients (91.6%) developed LMN sign by 4 years from symptom onset. ALSFRS-R changed from 40 ± 3 to 32 ± 4 in UMN-D ALS patients in 4 years ($t = 1.83$, $P < 0.05$). The amplitude of motor unit action potential (MUAP) of the first interosseus dorsalis was higher and the duration of MUAP was longer in UMN-D ALS patients than in PLS patients ((582.5 ± 30.7) μv *vs* (353.5 ± 21.5) μv; ($t = 1.87$, $P < 0.05$));((19.8 ± 2.3) ms *vs* (9.6 ± 1.3) ms; ($t = 1.85$, $P < 0.05$)).

Conclusions: UMN-D ALS patients characterized by more females, more cases with bulbar onset and faster progression than PLS patients, and electromyographic evidence of focal denervation.

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P229 ALS ENTERS THE WORLD OF BIG DATA: INITIAL DESCRIPTION OF AND RESULTS FROM THE PRO-ACT PLATFORM

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Keywords: research resource, dataset, clinical data

Background: Large datasets are critical for identifying statistically significant and biologically relevant observations, particularly for diseases resulting from the intricate interplay of genetic and environmental factors. The Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform provides an unprecedented opportunity to increase our understanding of the ALS patient population and the natural history of the disease.

Objective: To build, analyze, and make publicly available the world's largest merged dataset of patient clinical data from completed Phase II/III ALS clinical trials.

Design and methods: We obtained 6700+ patient records from 11 completed industry-sponsored tri

records from 7 government/non-profit sponsored trials. These data include the placebo arms from all trials and the drug arms from the majority of trials, including the riluzole trials. Utilizing empirical and theoretical knowledge from the literature, common data elements developed by NINDS and NEALS, and with input from clinical investigators, we have developed an ALS specific common data structure. We have used this common data structure to enable the merging of disparate datasets for the first time into a unified searchable dataset.

Results: The dataset contains at minimum an estimated 6 million datapoints, including never before described data, collected over a time period of 6–22 months (average 13 months). We have begun statistically analyzing this dataset so as to describe its contents and increase its utility for the research community. Baseline characteristics, survival distribution, and identified prognostic factors will be described. The rate of functional decline was compared between the subset of the industry-sponsored trials and the government/foundation-sponsored trials that assessed ALSFRS to ensure that the respective patient populations were similar. The full dataset will be made freely accessible to the research community for analysis and download (anticipated release Dec 2012 to coincide with ALS/MND), and a sample of the data will be released this summer for the ALS Prediction Prize challenge promoting the development of quantitative methods to predict future ALS progression.

Discussion: The PRO-ACT platform is the result of a unique collaboration among industry, academic, and foundation partners. Creation of this platform will enable a wide variety of analyses that were previously not possible. Use of this large merged ALS clinical dataset by the research community will help us better understand the natural history of the disease, further efforts to successfully stratify ALS patients, and provide insights into the design and interpretation of future clinical trials. Academic institutions and companies are encouraged to both contribute and analyze data for new insights into ALS. Pooling and analyzing clinical trial data is currently being explored for a wide variety of related diseases including Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, and Frontotemporal Dementia, and the PRO-ACT platform will serve as a model for these future efforts.

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P230 GRAY MATTER DEMYELINATION IS UNEXPECTEDLY COMMON IN NEURODEGENERATION: AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: NG2 glia, oligodendrocyte, myelination

Background: ALS is a non-cell autonomous disease as astroglia and microglia play a role in disease progression in rodent models. However, whether oligodendrocyte lineage is involved in ALS pathogenesis is not clear. Our recent study has shown that NG2 glia, also known as oligodendrocyte progenitor, proliferate and undergo dramatic changes in the lumbar spinal cord gray matter in G93A SOD1 mice at endstage. This suggests that oligodendroglia might degenerate during disease progression, which could be causing NG2 glial proliferation. More importantly, in ALS patients, although it is well known corticospinal tract demyelination occurs, it is largely unknown whether there are oligodendrocyte lineage changes in motor cortex and spinal cord gray matter where motor neurons degenerate.

Objectives: Given that NG2 glia are restricted to oligodendrocyte differentiation in ALS mouse lumbar spinal cord gray matter, we asked whether oligodendrocyte degenerate as disease progresses as measured by myelination in mouse. In addition, we investigated whether oligodendrocyte lineage undergoes similar changes in the CNS gray matter of ALS patients.

Methods: Immunohistochemistry (IHC) was used to determine NG2 glia cell morphology. IHC staining, luxol fast blue myelin staining and erichrome cyanine myelin staining were performed to evaluate myelin expression. Western Blotting was used to quantitatively evaluate myelin protein expression. MRI *in vivo* imaging was carried out to determine mouse lumbar spinal cord myelin signals.

Results: In ALS mouse lumbar spinal cord, loss of myelin basic protein expression/demyelination was seen as early as disease onset. Repeated longitudinal mouse MRI studies also suggested that gray matter demyelination occurred *in vivo* at the onset of clinical disease. Further myelin staining showed clear gray matter demyelination and distorted myelin structures in the lumbar spinal cord gray matter. In ALS patient motor cortex gray matter, NG2 glia showed increased immunoreactivity compared to those without neurological diseases. In addition, patchy demyelination in the gray matter of motor cortex and spinal cords was reliably and repeatedly observed, with preservation of neurons in the some regions of demyelination . Furthermore, we found that in ALS patients the decrease in myelin protein expression was correlated to oligodendrocyte progenitor marker, PDGFαR, expression, which is consistent with our animal studies.

Discussion: Both animal and human studies unexpectedly showed *gray matter* oligodendrocyte degeneration as measured by different myelin stains and *in vivo* MRI imaging in ALS. Since oligodendrocytes not only facilitate saltatory conduction of action potential, but also support neuronal functions such as lactate metabolism, through oligodendroglial specific MCT1, the injury to oligodendroglia in ALS may have pathogenic consequences.

Conclusions: Oligdendrocytes degenerate in the gray matter in ALS.

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P231 SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN AMYOTROPHIC LATERAL SCLEROSIS. RELATION TO ITS INCREASED EXPRESSION OF SKIN

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Keywords: skin, serum, vascular endothelial growth factor

Background: Vascular endothelial growth factor (VEGF), first discovered as a tumor-secreted protein that promotes vascular permeability, is an important angiogenesis factor

VEGF is expressed in many tissues and is rapidly upregulated during hypoxia, which occurs during acute or chronic vascular disease, pulmonary disease, or cancer. The precise mechanisms governing this upregulation remain unknown, but oxygen deprivation is a key regulator of VEGF gene expression with cells rapidly accumulating hypoxia-inducible transcription factors (HIFs) in response to decreased oxygen tension. A central role of VEGF in the pathophysiology of motor neuron disease has been raised by studies in mice where the hypoxia-response element of the VEGF promoter has been deleted. The VEGF165 isoform stimulates normal and abnormal vessel growth and promotes survival of motor neurons during hypoxia, VEGF gene expression is stimulated mainly through binding of HIF to a defined hypoxia-response element in the VEGF promoter.

Objectives: We measured serum levels of VEGF in ALS patients and controls, and studied the relationship between serum levels of VEGF and the density of VEGF immunoreactivity of skin in ALS patients.

Methods: Skin biopsy samples were taken from the left upper arm of 20 patients with ALS (mean age 62.1 years) and from 20 controls with other neurodegenerative diseases matched for sex and age (mean age 59.7 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. The sections were incubated with anti-VEGF antibody. The sections were stained by ABC kit. The immunoreactivity was quantified with an image-analysis system. Blood samples were collected from the same 20 ALS patients and the same 20 controls. Samples were analyzed using commercially available ELISA kits for VEGF.

Results: Serum VEGF levels were significantly higher ($p < 0.02$) in ALS patients (365.4 ± 140ng/l) than in controls (203.6 ± 125.9ng/l). There was a moderate positive relationship ($r = 0.51$) between duration of the disease and VEGF levels in serum from ALS patients. The immunoreactivity of VEGF was strongly positive in the epidermis and in some blood vessels and glands of the reticular dermis in all ALS patients. Its optical density in ALS patients (6.22 ± 2.91) was significantly higher ($p < 0.001$) than in controls (1.65 ± 0.61). Furthermore, there was a significant positive relationship ($r = 0.84$, $p < 0.001$) between the immunoreactivity and duration of illness in ALS patients. There was a significant positive relationship ($p < 0.02$) between serum VEGF concentrations and the optical density of VEGF in ALS patients.

Conclusions: The increased amount of serum VEGF may reflect in part the increased VEGF immunoreactivity of skin in ALS and may be involved in the pathogenesis of ALS.

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P232 A COMPARATIVE STUDY OF BIOMARKERS FOR DYSPHAGIA IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: dysphagia, swallowing, animal models

Background: Swallowing impairment (dysphagia) is common in amyotrophic lateral sclerosis (ALS), resulting in malnutrition, dehydration, and pulmonary aspiration. These symptoms are associated with a poor quality of life and contribute to death. No effective treatments currently exist for dysphagia in ALS.

Objectives: Our lab is dedicated to studying animal models of ALS to hasten the identification of novel treatments for dysphagia. Previous work in our lab showed that, like people with ALS, the SOD1-G93A transgenic mouse develops symptoms of dysphagia. Dogs with degenerative myelopathy (DM) exhibit a spontaneous SOD1 mutation that results in ALS-like symptoms of the limbs similar to humans and transgenic mice. Unspecified swallowing impairment was recently reported in end-stage dogs with DM. Our goal is to identify common biomarkers of dysphagia in ALS across species (mouse, dog, and human).

Methods: We have developed 4 dysphagia assays that have the potential for universal application in mammals: 1) Video analysis (VA) of oral ingestive behaviors, 2) Fluoroscopic (x-ray) analysis (FA) of swallowing, 3) Laryngeal brainstem response (LBR), and 4) Histological analysis (HA) of the brainstem nuclei involved in swallowing (trigeminal, facial, ambiguus, hypoglossal, dorsal motor nucleus of the vagus, and nucleus tractus solitarius). Using VA methodology, we investigated the lick rate of SOD1-G93A mice ($n = 14$) and non-transgenic littermates ($n = 12$) of either sex at 3, 5, and 7 weeks of age. For HA, brainstems of formalin-fixed end-stage DM ($n = 4$) and control ($n = 4$) dogs, and ALS ($n = 2$) and control ($n = 2$) humans were paraffin processed and stained using hematoxylin & eosin to identify brainstem nuclei and general histopathology by light microscopy. Researchers were blinded to genotype for all assays.

Results: At all time points, SOD1-G93A mice demonstrated a significantly slower lick rate compared to controls ($p < 0.05$). We are now establishing biomarkers of dysphagia in this strain using FA, LBR, and HA methods. In all canine DM and human ALS samples, subtle evidence of neurodegeneration was identified within each of the brainstem nuclei involved in swallowing. This finding is markedly different from our previous report of prominent vacuolization in the brainstem nuclei of SOD1-G93A mice.

Discussion and conclusions: Early-onset impairment of lick rate in SOD1-G93A mice suggests this strain may be a model of bulbar rather than spinal onset ALS, making it uniquely suitable for translational research to humans with bulbar onset ALS (˜30% of ALS cases). However, our histological findings suggest that neurodegeneration in canine DM may more closely recapitulate human ALS. We are currently testing this hypothesis using VA and FA methods to establish a clinicopathological correlation of dysphagia in canine DM for comparison with SOD1-G93A mice. Identification of common biomarkers of dysphagia in ALS across species is a critical step toward the identification of novel therapeutics suitable for pre-clinical trials.

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P233 NOVEL MARKERS OF MITOCHONDRIAL DYNAMICS IN AXONS ARE DISRUPTED IN MUTANT SOD1 TRANSGENIC MICE

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Keywords: mitochondrial fusion, axon, SOD1

Background: Distal axonopathy with loss of integrity of the neuromuscular junction occurs early in the pathogenesis of a familial form of ALS caused by mutations in SOD1 (ALS1). A strong and early mitochondrial phenotype also is observed in culture and transgenic mouse models, including altered morphology, fission/fusion, transport and bioenergetics. The dramatic rounding of mitochondria observed in cultured motor neurons expressing the ALS1-causing mutant, SOD1G93A, correlated with inhibition of mitochondrial fusion. This led us to investigate the role of post-translational modifications of profusion proteins and how they are affected by mutant SOD1.

Objectives: To assess differences in mitochondrial dynamics in neuronal cell bodies and axons and the impact of mutant SOD1 on these processes.

Methods: Explant cultures of murine dorsal root ganglia (DRG) were used for this study because of the ability to analyze cell bodies and axons separately by biochemical techniques. Mitochondrial fusion was assayed by co-expressing photoactivable EGFP targeted to the mitochondrial matrix. Profusion proteins were examined by Western analysis of extracts prepared from cell body and axonal compartments of the cultures and from brain, spinal cord and sciatic nerves of wildtype mice and those carrying SOD1WT or SOD1G93A transgenes.

Results: In cultured neurons, the rate of mitochondrial fusion was higher in the cell body than in the axon, suggesting a local regulation of profusion proteins which could be affected in ALS1. Full length Mfn2 was detected on Western blots as a 83 kDa band. However, in extracts of DRG axons and of mouse sciatic nerve and brainstem, this band was diminished and two smaller bands were labeled by antibody to Mfn2, migrating at 70 kDa and 13 kDa. This suggested the smaller forms (sMfn2) arose from proteolytic cleavage of full length Mfn2. Preferential cleavage in axons was indicated by relative absence of the sMfn2 in DRG cell bodies and in regions of nervous tissue dominated by cell bodies and dendrites (i.e., lumbar spinal cord, cortex or cerebellum). *In silico* analysis identified a presenilin-like consensus sequence in the predicted cleavage region. Interestingly, these sMfn2 were absent from sciatic nerve of SOD1G93A transgenic mice at 120 days of age, just prior to appearance of overt symptoms.

Discussion: The data provide strong evidence of specialized processes controlling mitochondrial dynamics in neuronal cell bodies and axons. Cleavage of the profusion protein, Mfn2, in axons correlated with reduced rate of mitochondrial fusion. Absence of Mfn2 cleavage in sciatic nerve of fALS1 transgenic mice implicates abnormal protease activity in the axonopathy that is an early feature of the disease. We propose that sMfn2 could serve as a biomarker for evaluation of therapeutic efficacy.

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THEME 11 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

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JR Williams, N Atassi, I Nakano, JS Mora, V Drory, M Cudkowicz, J Shefner, A Chiò, H Mitsumoto, V Silani, L Zinman, M Weber, LH Van Den Berg, W Robberecht, L-O Ronnevi1, O Hardiman, L Bruijn, D Kerr

P235 HEART RATE VARIABILITY CORRELATES ALSFRS-R IN AMYOTROPHIC LATERAL SCLEROSIS
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C Gibbons, E Thornton, J Ealing, D Mitchell, P Shaw, K Talbot, A Tennant, C Young

P238 DISEASE SEVERITY AND DISEASE TRAJECTORY OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS AT FIRST CLINIC VISIT MEASURED PROSPECTIVELY WITH "ALS DASHBOARD" - A SIX-DOMAIN (COGNITION, AFFECT, BULBAR, RESPIRATORY, ARM, LEG) STAGING SYSTEM
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P243 VALIDATION OF THE SIX MINUTE WALK TEST (6MW) IN AMBULATORY AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS
MS Sanjak, VL Langford, SE Holsten, J Bockenek, EK Bravver, UG Desai, WL Bockenek, NM Williams, PC Russo, BR Brooks

P244 PROSPECTIVE LONGITUDINAL VALIDATION OVER TIME OF CLINICAL DISEASE TRAJECTORY (CDT) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS BY 'ALS DASHBOARD' CONTAINING COGNITIVE – BEHAVIOR (DEPRESSION – PSEUDOBULBAR AFFECT (PBA) – BULBAR – RESPIRATORY – ARM – LEG DOMAINS – RATE OF LEG AND ARM DOMAIN CLINICAL STAGE CHANGES PER PATIENT IS STATISTICALLY GREATER THAN RESPIRATORY OR BULBAR STAGE CHANGES
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P248 SIGNIFICANT CMAP DECREMENT BY REPETITIVE NERVE STIMULATION IS MORE FREQUENT IN MEDIAN THAN ULNAR NERVES OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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P250 ALS AND VEGF: MODULATION BY NON-INVASIVE VENTILATION

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P251 LONGITUDINAL CHANGES OF OUTCOME MEASURES IN SPINAL AND BULBAR MUSCULAR ATROPHY

A Hashizume, M Katsuno, H Banno, K Suzuki1, N Suga, T Mano, N Atsuta, H Oe, H Watanabe, F Tanaka, G Sobue

THEME 11 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P234 DEVELOPMENT OF A "RED FLAGS" SYMPTOM LIST TO ASSIST WITH EARLY RECOGNITION AND DIAGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Red flag, symptoms, diagnosis

Introduction: Amyotrophic lateral sclerosis (ALS) diagnosis is primarily based on physician-observed signs and symptoms. Currently, there is a median delay of 8–16 months from symptom onset to diagnosis, mainly due to similarities between early symptoms of ALS and other common, non-fatal, diseases. Identifying which symptoms are most predictive of ALS may decrease diagnostic delay. This initiative aimed to develop a shortlist of hallmark symptoms to assist with early recognition/ diagnosis of ALS.

Methods: A shortlist of characteristic ALS signs and symptoms for ALS diagnosis was developed at a meeting of ALS specialists (June 2011). This shortlist, termed 'ALS red flags', was developed by the ALS specialists based on their clinical experience and a review of their patient databases. The ALS red flags list is being validated using patient data from the General Practice Research Database (GPRD), which contains anonymised data from ~630 primary care practices (over 11 million patients) in the United Kingdom. Published literature indicates that the computer-identified ALS incidence from the GPRD is comparable with rates in population-based registries in Scotland, Ireland, and Italy; thus this is a reasonable database for validation of the red flags list. Red flags will be abstracted from the medical records of approximately 2000 patients with ALS referred to neurologists by general practitioners (GPs). Latent class analyses will test if red flag clusters can differentiate patients with ALS from other neurology referrals.

Results: Hallmark ALS symptoms include clumsiness, speech change, dysarthria and dysphagia, cramps, fatigue, and gait changes; signs include fasciculation, atrophy, and weakness. Potential modifying terms include progressive, excessive, persistent or unusual location. Examples of candidate ALS red flags are 'excessive fatigue', 'persistent cramps', 'tongue fasciculation', or 'unusual location weakness' (not adhering to radicular or peripheral nerve distribution). Results from the ongoing validation of the red flags list will be presented.

Conclusions: It is expected that the ALS red flags list will enable clinicians, particularly GPs and neurologists, to recognise ALS signs and symptoms at an early stage. This will facilitate appropriate referral, and may lead to earlier treatment and improved outcomes.

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P235 HEART RATE VARIABILITY CORRELATES ALSFRS-R IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: heart rate variability, autonomic dysfunction, ALS functional rating scale-revised

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons, however it is increasingly recognized that non-motor manifestations may occur, including autonomic nervous system dysfunction. Therefore, our first objective in these studies was to evaluate autonomic dysfunction in patient with ALS.

Objectives: Eighty-two patients with sporadic ALS were compared to 172 healthy controls.

Methods: Heart rate variability (HRV) and ALS functional rating scale-revised (ALSFRS-R) was collected in ALS patients.

Results: A decrease in HRV was found in the ALS patients, indicating dysfunction of autonomic cardiac control ($p < 0.0001$). ALSFRS-R was positively associated with HRV ($p, 0.05$).

Discussion: These results suggest that cardiac autonomic dysfunction in patients with ALS has sympathetic-activated

balanced with decreasing both sympathetic and parasympathetic components. HRV was found to be related to disability of the disease.

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P236 COMPARISON OF AN AMYOTROPHIC LATERAL SCLEROSIS STAGING SYSTEM WITH THE REVISED AMYOTROPHIC LATERAL SCLEROSIS FUNCTIONAL RATING SCALE

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Keywords: ALS Staging System, measuring disease progression, ALS-FRSr

Background: A disease staging system is a set of milestones occurring in a specified order, representing progression through a disease. A staging system has been proposed for amyotrophic lateral sclerosis (ALS) consisting of four stages occurring at predictable proportions of the disease course: Stage 1 (first region involved i.e. symptom onset), Stage 2A (diagnosis), Stage 2B (second region involved), Stage 3 (third region involved), Stage 4A (need for gastrostomy) and Stage 4B (need for non-invasive ventilation)(1). An established measure of functional decline in ALS is the revised ALS Functional Rating Scale (ALS-FRSr). While a functional scale is not a staging system, the ALS-FRSr nonetheless has a relationship with disease progression. The two systems have some similarities. ALS-FRSr questions reflect regional involvement, corresponding to Stages 1–3 and need for gastrostomy or non-invasive ventilation. ALS-FRSr score < 5 indicates Stage 4B has been reached and a score < 7 indicates Stage 4 has been reached.

Objectives: The aim was to compare ALS Stage with ALS-FRSr in a cohort of patients.

Methods: A database of ALS patients from ten UK centres who had participated in a clinical trial between 2009 and 2011 was analysed. The ALS Stage and the concomitant ALS-FRSr score were recorded for each patient visit to the trial at baseline, week 12 and months 6, 9, 15 and 18. Median, range and interquartile range of ALS-FRSr were calculated for each ALS Stage.

Results: There were 217 patients included in the study. 78.3% had limb onset disease and 21.7% had bulbar onset disease. 69.6% were male patients and 30.4% were female patients. ALS-FRSr scores were not normally distributed. The median ALS-FRSr scores with interquartile ranges at each stage were Stage 2A: 43.0 (41.0–45.0), Stage 2B: 38.0 (35.0–42.0), Stage 3: 34.0 (29.5–37.5), Stage 4A: 25.0 (19.25–31.75) and Stage 4B: 23.0 (18.00–29.25). Patients with bulbar onset ALS had higher ALS-FRSr scores at Stage 4A with a median of 29.0 (22.25–33.75) whilst patients with limb onset ALS had lower scores at Stage 4A with a median of 19.0 (16.5–25.5).

Discussion: The median ALS-FRSr score should decline as ALS progresses, and the spread of ALS-FRSr scores should widen since not all patients are equally functionally impaired, depending on the pattern and type of weakness. We observe both these effects, confirming that the ALS staging system used is a measure of disease progression. Limb onset patients are more functionally impaired than bulbar onset patients by the time they reach Stage 4A.

Conclusions: The staging system measures progression through the disease while the ALS-FRSr reflects overall functional decline. The staging system is a useful additional measure which can be utilised in patient care and ALS research.

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P237 RASCH ANALYSIS OF THE AMYOTROPHIC LATERAL SCLEROSIS FUNCTIONAL RATING SCALE–R (ALSFRS-R)

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Keywords: ALSFRS-R, rasch, validation

Background: The ALSFRS-R is the gold standard questionnaire measure of functional impairment in patients with ALS. The scale is widely used in clinics and research although its measurement properties, beyond internal consistency and construct reliability, have not been examined.

Objective: To apply ALSFRS-R data to the Rasch model in order to rigorously evaluate the psychometric properties of the scale and assess the scale's ability to produce interval-level measurement.

Methods: A questionnaire pack containing the ALSFRS-R was sent to 147 consecutive patients with a confirmed diagnosis of MND.

Results: The ALSFRS-R displayed marginal fit to the Rasch model ($\chi^2_{(24)} = 37.80$, $p = 0.04$). Item 9 "How has your ability to climb stairs changed?" displayed a high fit residual ($+3.34$ logits) indicating possible irrelevance of the item. T-test analysis revealed that the 12-item scale displayed unacceptable multidimensionality (12.41% (CI 7.53–19.11%) significant tests). A modified 11-item version of the ALSFRS-R was examined following the removal of item 9. The modified scale displayed improved fit statistics including excellent fit to the Rasch model ($\chi^2_{(22)} = 29.64$, $p = 0.12$) high reliability (PSI = .91) and unidimensionality (8.97% significant T-tests (CI 4.86–14.84)).

Discussion: The 12-item ALSFRS-R scale does not satisfy the demands of the Rasch model. Multidimensionality present in the commonly-used summary score impacts upon the scale's ability to provide accurate comparisons between patients or over time. The modified ALSFRS-11 has excellent fit characteristics and is unidimensional. The ALSFRS-11 is therefore capable of producing interval level measurement, unlike the ALSFRS-R.

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P238 DISEASE SEVERITY AND DISEASE TRAJECTORY OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS AT FIRST CLINIC VISIT MEASURED PROSPECTIVELY WITH "ALS DASHBOARD" - A SIX-DOMAIN (COGNITION, AFFECT, BULBAR, RESPIRATORY, ARM, LEG) STAGING SYSTEM

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Keywords: disease burden, disease trajectory, benchmarking

Background: While the time from disease onset to disease diagnosis has been studied in several populations of people with ALS, the disease burden at first clinic visit and at diagnosis has been less carefully analyzed.

Objectives: Determine prospectively the distribution of clinical stages in people with ALS, at the time of first clinic visit and diagnosis according to the "ALS Dashboard" staging algorithm.

Methods: From 2009–2011 181 patients (101 M; 80 F) were referred to the Carolinas Neuromuscular/ALS-MDA Center. 17 were found not to have ALS. The remaining patients were staged according to the "ALS Dashboard" staging algorithm in cognitive, affect, bulbar, respiratory, arm and leg domains. Age at diagnosis, site of onset, time from onset to diagnosis, vital capacity at diagnosis, ALSFRS-R total and bulbar, arm, leg, respiratory subscores at diagnosis were calculated. Domain-specific stage at diagnosis and at three month intervals after diagnosis were established for each patient. Statistical analysis was performed with MedcCalc software version 12. 2.1 (www.medcalc.org/)

Results: Cognitive stage > 3 was present at diagnosis in 12.3%. Affect stage > 3 was present in 18.2%. Bulbar stage > 3 was present in 20.2%. Respiratory stage > 3 was present in 15.5% but increased to 70.6% by 3 months based on introduction of non-invasive ventilation. Arm stage > 3 was present in 23.6% at diagnosis. Leg stage > 3 was present in 42.4% at diagnosis and increased to 68.6% at 6 months. Bulbar stage changes were more rapid in bulbar onset patients than spinal onset patients. Lost to follow-up occurs at 2.5% at 3 and 6 months.

Conclusions: "ALS Dashboard" staging changes in this prospectively evaluated cohort was more rapid in the respiratory domain due to changes in standard of practice for these patients.

Discussion: Further analysis of ALS staging is required to understand the effect of lost to follow-up on natural history studies in patients with ALS.

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P239 RESTING AND EXERCISE-RELATED OXIDATIVE STRESS MARKERS IN PATIENTS UNDER ERYTHROPOIETIN TREATMENT

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Keywords: oxidative stress, exercise, erythropoietin

Background: Treatment with erythropoietin (EPO) has been suggested as possible therapeutic approach in ALS. Among the possible neuroprotective effects of EPO influence on cell oxidative stress balance has been suggested. The possible pathogenic role of oxidative stress on motor neurone degeneration in ALS is emphasized by a bulk of evidence derived from genetic, molecular and animal models. Furthermore, research interest has recently developed on the relationships between exercise, oxidative stress and motor neurone degeneration in ALS.

Objectives: We aimed to analyse the effect of EPO on exercise related oxidative stress parameters in ALS.

Methods: Along the currently ongoing Italian multi-center, double blind, randomized, placebo-controlled "EPOS" clinical trial for safety and efficacy of erythropoietin in ALS, we analyse, in a group of patients, some peripheral oxidative stress biomarkers (advanced oxidation protein products-AOPP, ferric reducing ability of plasma- FRAPP and total glutathione) basally and at 3, 6 and 9 months during the administration of either EPO or placebo. We also analysed the modifications of these parameters with aerobic exercise (cycloergometer or handgrip incremental workload test), performed by a subset of patients.

Results: At this moment of writing the abstract, our still blinded data show, before treatment, increased levels of oxidative stress parameters in ALS patients, compared to age-matched controls. After treatment it is possible to distinguish two subgroups of patients: the first group showing a trend of increment of AOPP levels and reduced levels of total glutathione, indicating increased oxidative stress, while the second group shows no differences in these parameters.

Discussion and conclusion: The opening of blindness at the end of the study will allow us to relate laboratory to clinical data, in order to better interpret this data. This will be useful to clarify the oxidative stress modulation occurring during aerobic exercise and verify a possible modifying role of erythropoietin on the oxidative stress balance.

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P240 QUANTITATIVE LABIAL, TONGUE SPEECH AND SWALLOWING RATE MEASUREMENTS AT DIAGNOSIS CORRELATE WITH ALS FUNCTIONAL RATING SCALE – REVISED (ALSFRS-R) BULBAR SUB-SCORE AND BULBAR SUB-SCORE ITEM SCORES BUT NOT WITH ALSFRS-R TOTAL SCORE – INTER-RATER RELIABILITY IN SPEECH AND SWALLOWING DOMAINS

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Keywords: bulbar function, oral motor, benchmarking

Background: Labial articulation rate (LAR) and tongue articulation rate (TAR) (words-per-minute (WPM) or words-per-minute-per-breath (WPMPB)) changes over time significantly prior to intelligibility changes. Liquid swallowing rate(LSR)(mL-per-swallow-per-minute (mLPSPM) or mL-per-swallow-per-minute-per-breath(mLPSPMPB)) changes prior to video fluoroscopic swallowing changes and predicts oral feeding safety capacity across a wide-range of causes of neurogenic dysphagia. Quantitative changes in LAR, TAR and LSR allows assessment of disease progression in the speech and swallowing domains of bulbar function and may allow assessment of response to pharmacologic or physical treatments in prospective clinical trials.

Objectives: Determine the LAR and TAR at diagnosis and compare with LSR in the same ALS-MND patients. Determine inter-rate variability for LAR, TAR and LSR in a subgroup of ALS-MND patients at diagnosis. Determine the relationship of LAR, TAR and LSR to ALSFRS-R total score and ALSFRS-R bulbar subscore total and bulbar sub-score speech, salivation and swallowing item-score.

Methods: LAR was measured by repeating "pepper" and TAR was measured by repeating "ticker" 10 times and recording the start and completion time in seconds. The number of breaths required to complete the task were recorded. LSR was measured by recording the time for swallowing 90 mL(3oz) water from the initiation of liquid leaving the cup at the lips to the completion of the final swallow of liquid in seconds. The number of breaths required to complete the task were recorded. Examiner attention is required to note that the final swallow in some patients may be delayed after the liquid enters the mouth. Qualitative properties, cough, clearing throat, spurting out liquid from mouth or nose, either during or after swallow test were recorded. Wetness of voice quality at completion of swallow test was also recorded. WPM, WPMPB, mLPSPM, and mLPSPMPB were calculated and statistical analysis was performed with MedcCalc software version 12. 2.1 (www.medcalc.org/)

Results: At diagnosis in 118 ALS-MND patients LAR, TAR and LSR were measurable in 104 patients. LAR, TAR, LSR measured as WPM and mLPSPM distributed differently than LAR, TAR, LSR measured as WPMPB and mLPSPMPB and were not normal. LAR and TAR correlated significantly across patients ($R2 = 0.995$;$p < 0.001$). LAR and TAR correlated significantly with LSR across patients ($R2 = 0.762$; $R2 = 0.883$: $p < 0.01$). Inter-rater reliability in 36 paired tests was excellent (Spearman rho LAR = 0.889; TAR = 0.881; LSR = 0.773;$p < 0.01$). LAR, TAR, LSR did not correlate with ALSFRS-R total score. LAR ($R2 = 0.891$) or TAR($R2 = 0.848$) significantly correlated($p < 0.01$)with ALSFRS-R bulbar sub-score speech item score. LSR($R2 = 0.788$)significantly correlated($p < 0.01$)with ALSFRS-R bulbar sub-score swallowing item-score.

Conclusions: LAR, TAR and LSR are robust quantitative bulbar clinimetrics that provide specific outcome measurements correlating with bulbar functional milestone scales in the ALSFRS-R but not with the ALSFRS-R total score.

Discussion: LAR, TAR, LSR will provide more precision in assessing response to treatment in speech and swallowing domains of patients with ALS.

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P241 VALIDATION OF ROBUST TOOLS TO MEASURE SIALORRHEA IN ALS: A STUDY IN A LARGE FRENCH COHORT OF ALS PATIENTS

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Keywords: sialorrhea, scale, reliability

Background: Sialorrhea is a frequent and disabling problem in patients with bulbar involvement related to amyotrophic lateral sclerosis (ALS). There is an unmet need for validated tools to measure sialorrhea in ALS, especially to evaluate treatments.

Objective: We assessed prospectively the inter/intra rate reviewer reliability of two scales: the Oral Secretion Scale (OSS), specifically developed for ALS patients (1,2), and the Sialorrhea Scoring Scale (SSS), initially developed for Parkinson disease patients (3,4).

Methods: Sialorrhea was rated in 69 ALS consecutive patients by four evaluators: two neurologists, one nurse and one speech therapist. Inter-rater reliability was evaluated by the light kappa coefficient and intra-rater reliability by the weighted kappa coefficient. We also compared patients' and caregivers' answers.

Results: The two scales present a high inter/ intra-rater reliability: weighted kappas were 0.85 for both scales and light kappas 0.89 for the OSS and 0.88 for the SSS. Both scales also showed a good intra-profession reliability (OSS kappa = 0.84; SSS kappa = 0.79) and agreement between patients' and caregivers' answers. The SSS showed a higher responsiveness compared to OSS.

Discussion: Our population was representative of a standard ALS population since we prospectively included all the ALS patients attending a multidisciplinary evaluation during the study period. From a practical point of view, the two questionnaires are rapid to administer and do not exceed 5 minutes. Given the very good agreement between patients' and caregivers' answers in the intra-rater test, we can practically assess saliva retention, using one either of the two scales OSS or SSS, by phone and /or by contacting the caregiver especially when the patient is dysarthric or in advanced stage of his disease. This point is an advantage for the monitoring of treatment's effect and for therapeutic trial.

Conclusion: Both OSS and SSS are reliable tools to measure sialorrhea in ALS patients. Because of the wide range of salivation degrees, SSS may be more sensitive to evaluate treatments in patients with severe hypersialorrhea.

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P242 MOTOR FATIGUE SIGNS ARE NOT PRESENT DURING THE SIX MINUTE WALK TEST (6MWT) IN AMBULATORY AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Keywords: 6 minute walk test, motor fatigue, velocity

Background: The 6 minute walk test (6MWT) has been shown to be a reliable measure of motor function and correlated with disability in neuromuscular disorders (1,2,3). We previously validated the 6MWT with other outcome measures that are commonly used to evaluate motor function and impairment in ALS. We have shown that the 6MWT distance significantly correlated with ALSFRS-R total score, lower extremity muscle strength, the 25 foot walk test, timed up and go, and number of falls but did not correlate with FVC (3). A concern was that ALS patients may experience motor fatigue during the 6MWT which could compromise its internal validity.

Objectives: Evaluate signs of motor fatigue defined as rate of decline in walking distance and velocity during the 6MWT in ambulatory ALS patients.

Methods: Walking distance was recorded in 2 minute intervals during a standardized 6MWT. Fifteen ambulatory ALS patients (9 males, and 6 females, age = 65 ± 11) performed the 6MWT according to modified American Thoracic Society (ATS) Protocol (4). Participants were instructed to walk as far as possible for 6 minutes on a flat, hard surface, wearing shoes, and using their usual assistive devices in a 25 meter hallway. Participants were instructed to stop and rest during the test as needed then resume walking as soon as they could until the 6 minutes was completed. Walking velocity was calculated from distance recorded at 2, 4, and 6 minutes.

Results: Mean ± SD distance recorded at 2, 4, and 6, were similar in the three intervals (127 ± 29, 116 ± 27,125 ± 41 meters respectively, ANOVA p = 0.6499). Correspondent gait velocities were also similar (1.05 ± 0.24, 1.02 ± 0.22, and 1.02 ± 0.24 m/sec).

Discussion and conclusions: Our preliminary data indicates that fatigue defined as decline in walking distance and speed during the 6MWT is not present in ambulatory ALS patients. This is similar to observations obtained from multiple sclerosis patients (1) and in contrast with spinal muscular atrophy patients who exhibited significant fatigue by the decline in distance from first to sixth minutes of 6MWT (2). Therefore, our previous conclusion that the 6MWT is a robust outcome measure expanding the dynamic range of treatment measurement in ambulatory ALS patients early in disease progression seems to be confirmed.

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P243 VALIDATION OF THE SIX MINUTE WALK TEST (6MW) IN AMBULATORY AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Keywords: outcome measure, motor function, validation

Background: Evaluation of motor impairments in ALS patient is necessary for clinical decision making, natural history, and efficacy of therapeutics. The recent surge in therapeutics with potential to improve rather than reduce progression of motor function in ALS may require a sensitive and meaningful functional outcome measure. 6MWT is being increasingly used in a variety of neurodegenerative motor system diseases including spinal muscular atrophy and ALS.

Objectives: To validate 6MW with outcome measures frequently used to evaluate motor performance in ALS which include the ALS Functional Rating Scale-Revised (ALSFRS-R), Quantitative Muscle Test (QMT), forced vital capacity (FVC),

4-step stair climbing(SC), 25-foot walk test(25FWT), timed-up-and-go(TUG), and number of falls(F).

Methods: Distance during the 6MW, ALSFRS-R, FVC, 25FWT, TUG, F, and QMT of bilateral hip, knee flexors and extensors, and ankle dorsiflexors were evaluated in 56 ambulatory ALS patients following standard procedures. TUG was evaluated as the time it took the patient to stand up from standard chair, walk 3 meters, and return to the chair. SC and 25FWT were evaluated as the time it took the patients to go up and down 4 steps and walk 25 foot respectively. Pearson's correlations were used to test for linear relationships between the outcome measures.

Results: 6MW distance significantly correlated with ALSFRS-R total score($r = .49$;p0.002), QMT($r = .58$;p0.02), SC($r = -.57$; p 0.001), 25FWT($r = -.51$;p 0.005), and TUG($r = -.65$; p 0.001), but did not correlate with FVC($r = .18$ p 0.16). In addition, 6MWT significantlly correlated with number of falls, F($r = .42$;p 0.002)experienced by ALS patients.

Discussion and conclusions: 6MW correlates with other outcome measures that quantify lower extremity motor deficit in ALS, but does not correlate with FVC. 6MW is a robust outcome measure expanding the dynamic range of treatment measurement in ambulatory ALS patients early in the disease. 6MWT correlates with the number of falls which is a meaningful safety-related outcome measure for ALS patients.

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P244 PROSPECTIVE LONGITUDINAL VALIDATION OVER TIME OF CLINICAL DISEASE TRAJECTORY (CDT) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS BY 'ALS DASHBOARD' CONTAINING COGNITIVE – BEHAVIOR (DEPRESSION – PSEUDOBULBAR AFFECT (PBA) – BULBAR – RESPIRATORY – ARM – LEG DOMAINS – RATE OF LEG AND ARM DOMAIN CLINICAL STAGE CHANGES PER PATIENT IS STATISTICALLY GREATER THAN RESPIRATORY OR BULBAR STAGE CHANGES

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Keywords: cannonical staging, ALS Dashboard, benchmarking

Background: CDT is currently evaluated per-patient by ALS Functional Rating Scale-Revised (ALSFRS-R) without attention to cognitive and behavioral (depression/pseudobulbar affect-PBA) domains that are not included in current proposed staging algorithms.

Objective: Assess CDT with 'ALS Dashboard' longitudinally to define differences in rate of clinical stage changes in different domains.

Setting: Multidisciplinary ALS Clinic.

Methods: ALS patients(99/263), categorized as El Escorial Criteria Clinically Definite (EECD)(90) or Awaji-Shima Criteria Clinically Definite (ASCD)(109) were staged longitudinally according to ALS Dashboard criteria.

Results: At diagnostic visit in EECD/ASCD ALS > stage-3 cognitive (8.9% / 13.8%) and respiratory dysfunction (7.8% / 4.6%) was similar, but > stage-3 PBA (11.1% / 2.8%; $p = 0.0400$), depression (25.6% / 10.0%; $p = 0.007$), bulbar-dysfunction (45.6% / 13.8%; $p = 0.0001$), arm-dysfunction (35.6% / 8.3%; $p = 0.0001$), leg-dysfunction (55.6% / 33.9%; $p = 0.0040$) were significantly increased in EECD-ALS. ALS Dashboard identifies earlier development of Affect-Bulbar-Arm-Leg, but not Cognitive-Respiratory > stage-3 disease in EECD- than ASCD-ALS patients with subsequent faster progression in EECD-ALS patients. Development of increased Leg clinical stages ($0.22 + 0.09$ (SD) stages per patient per 3 months) was identical with increased Arm clinical stages (0.18 ± 0.10) but statistically increased ($p = 0.0495$) compared with increased Bulbar clinical stages (0.10 ± 0.09) and increased Respiratory clinical stages (0.03 ± 0.03) post diagnostic visit.

Conclusion: ALS Dashboard is a novel tool for analyzing accumulation of disease severity within a single patient and across different patients. ALS severity in some, but not all domains, segregates differently with a higher proportion of > stage-3 Leg-Arm-Bulbar disease in EECD than ASCD ALS. The prospective rate of development of advanced leg and arm stages was statistically greater than the development of bulbar and respiratory stages. ALS Dashboard provides description of ALS milestone changes that permits more precision in comparing domains involved than rates of disability measured by ALSFRS-R.

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P245 AMYOTROPHIC LATERAL SCLEROSIS AND PREGNANCY

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Keywords: pregnancy, delivery

Nowadays amyotrophic lateral sclerosis (ALS) is registered in the young more often. Therefore, perhaps in the future neurologists and obstetricians-gynecologists will have to deal with pregnancy in patients with ALS more often.

We describe a case of pregnancy in ALS, registered in the Ukraine, and the peculiarities of ALS course during this period.

The female patient (32 years old), who has 2 children (14 and 4 years old), was admitted in INPN NAMSU with ALS diagnosis in March 2012 for a planned dynamic observation. A verified ALS (24 points on ALS FRSR) was diagnosed in INPN NAMSU in October 2011. The first signs of the disease appeared in September 2011 after discontinuation of breast feeding. The patient's height is 162 cm and weight is 49.5 kg. From 2010 to 2011 her weight loss was > 10 kg. The patient's complaints included speech changes, periodical choking

episodes of night difficulties of breathing, weakness in extremities (uses wheelchair and constant assistance of other person), menstrual disorders, twitches in all the body. In the neurological status a mild facial hypomimia, atrophy of tongue muscles with fibrillations, dysarthria, tetraparesis, fasciculations without sensory impairments was registered. The vital lung volume (VLV) was 24%. Patient's abdomen was increased although the patient did not attach any importance for that. An urgent gynecological consultation and ultrasound examination of the uterus confirmed a pregnancy at the term of 20 weeks. The family denied a proposal of a justifiable abortion. According to a psychological examination the patient's cognitive functioning was normal. Hematology and blood biochemistry parameters were normal. Results of viral hepatitis and herpetic infection markers analyses were negative. The patient is under dynamic observation by a neurologist, gynecologist, and anesthesiologist. Since 21 week of pregnancy she uses "Bipap ST" for 10–15 min before sleep and takes NeuroAid medication with unproven efficacy in ALS nor in pregnancy. Ultrasound examination on 21st week of pregnancy demonstrated no pathological changes in the fetus. During the period of observation the patient's weight gain was 6 kg,VLV increased up to 40%, SpO_2 was 96%. A range of movements has raised and the patient is able to move with assistance. The patient has no complaints concerning choking and her dysarthria becomes milder. In the neurological status tetraparesis with amyotrophic set of symptoms and fasciculations are registered. The term of delivery (probably 37–38 weeks) will be determined in accordance with the patient's conditions (VLV and SpO_2). The method of delivery management (Cesarean or vaginal) is not defined yet. If necessary, usage of an infiltrative anesthesia will be planned.

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P246 EYE MOVEMENT DISTURBANCE IS ASSOCIATED WITH COGNITIVE IMPAIRMENT IN ALS

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Keywords: eye movementes, dementia, gaze disturbance

Background: Although ocular motor movements have been considered spared in amyotrophic lateral sclerosis (ALS) for long time, their abnormalities are increasingly recognized in patients with ALS (1).The most frequent oculomotor dysfunction reported is ophthalmoparesis, particularly in tracheotomized longer-surviving patients, but also pursuit, saccadic, and nystagmus impairment has been described. However, there is not definite data about their frequency. Only single cases have been reported in literature about occurrence of eye movement disorders in ALS-plus syndrome, but systematic studies concerning this issue are lacking.

Objectives: To evaluate the frequency of gaze disturbance and its association with cognitive impairment in an ALS cohort.

Methods: We retrospectively analysed the occurrence of eye movement disorders and cognitive impairment in 300 ALS patients (aged 27–83 years), referred to our ALS Center.

Results: 14% of our patients (42/300) presented a gaze disturbance. The upward gaze limitation and the saccadation of pursuit were the most frequent disorders observed, isolated or associated. Three patients presented oculomotor apraxia and other three patients had a ptosis. Slow saccades and saccadic intrusions were also observed. One of the five tracheotomized patients included presented slow saccades. 50% (21/42) of ALS patients with oculomotor disorders had dementia, while only 8% of ALS patients without gaze disturbance were demented. Seven not demented patients with oculomotor disturbance showed an extra moto neuronal involvement, namely an extrapyramidal syndrome in 5 cases, a cerebellar syndrome in 1 case and dysautonomia in the last one.

Discussion: ALS is a neurodegenerative disorder primarily affecting the upper and lower motor neurons. Increasing evidence about an involvement of cerebral extra-motor areas in ALS is emerging. Neuroimaging, pathologic and neuropsychological studies together with genetic findings suggest that ALS doesn't affect the motor system exclusively, but rather should be considered part of a continuum clinico-pathological spectrum of disease, including frontotemporal dementia (FTD) with TDP-43 and FUS-proteinopathies. Considering the complex network at the basis of the voluntary gaze control, its exploration provides valuable information about various cortical and subcortical regions, besides the motor function. Oculomotor function may be impaired in ALS, despite the characteristic resistance of oculomotor nuclei, through frontal and parietal lobes involvement, in particular in ALS-FTD.

Conclusions: In our cohort we found eye movement impairment in 14% of patients, significantly more frequently in subjects affected by ALS-FTD than in not demented ALS patients ($p < 0.0001$). Therefore, the analysis of gaze disturbance and its early detection might be predictable of a cognitive involvement, thus assuming a negative prognostic significance. Further studies are needed and we intend to analyze the possible association between occurrence of eye movement disorders, FTD, and known mutations in genes causing ALS.

Reference

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P247 METABONOMIC ANALYSIS OF CEREBROSPINAL FLUID (CSF) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Keywords: metabonomics, biomarkers

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with an incidence of 2–5/100000 in France. Pathophysiological mechanisms involved in this disease are complex but remain for the most part unknown. This lack of knowledge might explain the absence of reliable biological markers in ALS. NMR spectroscopy (^{1}H NMR), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS) are powerful analytical methods providing a rapid metabolic fingerprint. These methods are considered as references to perform metabonomics approach. Some authors have previously shown that ^{1}H NMR and GC-MS could be relevant tools to explore CSF from ALS patients. However, all these tools have never been used together to explore the CSF metabolic profile of ALS patients. Moreover, biomarker specificity has never been tested using the same approach in different neurological diseases.

Objectives: The aim of this study was to analyze CSF of patients with ALS and different neurological diseases by ^{1}H NMR, GC-MS and LC-MS/MS in order to identify specific biomarkers in the early stage of ALS disease, and to identify some biochemical pathways involved in this disease.

Methods: CSF samples were collected from patients with ALS at the time of diagnosis (group I) and from patients with other neurodegenerative diseases (group II), multiple sclerosis (group III), chronic inflammatory demyelinating polyradiculoneuropathy (group IV) or peripheral neuropathy (group V). One and two-dimensional ^{1}H NMR, GC-MS and LC-MS/MS analyses were performed using "bucketing" methodology. We have just begun to independently analyze the data and check the coherence of relevant identified metabolites. PCA and OPLS-DA analyses were performed using Simca P + software. The quality of the model was described by R^2 and Q^2 values. Then, we focused on relevant metabolites using univariate statistical analysis.

Results: We analyzed 80 CSF samples from ALS patients and 23, 21, 18 and 30 CSF samples from groups II, III, IV and V, respectively. We identified more than 100 metabolites using ^{1}H NMR and MS such amino-acids and organic acids. At this date, we analysed 70% of the samples and others are currently being analysed. Multivariate analysis showed similar results using all analytical methods. Preliminary results of PCA (group I vs V) revealed that the two first PCs explained more than 55 % of the variation in the selected metabolites. OPLS-DA showed that both populations were separated with a $R^2 > 0.7$ and a $Q^2 > 0.3$. We noted that metabolites contributing to the separation of the two clusters are involved in aerobic glycolysis.

Conclusions: CSF screening by ^{1}H NMR spectroscopy and MS could be a good, rapid and easy tool to identify CSF metabolic profile and to suggest some hypotheses about pathophysiological mechanisms. Further experiments should help us to increase the robustness of the model, particularly using an external validation method.

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P248 SIGNIFICANT CMAP DECREMENT BY REPETITIVE NERVE STIMULATION IS MORE FREQUENT IN MEDIAN THAN ULNAR NERVES OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: repetitive nerve stimulation, early diagnosis

Introduction: Several studies have shown a significant amplitude decrement in compound muscle action potentials (CMAP) on repetitive nerve stimulation (RNS) of muscles involved in ALS. In ALS, muscle wasting preferentially affects the thenar muscles (APB) rather than the hypothenar muscles (ADM).

Methods: We performed RNS studies in the APB and ADM muscles of 32 ALS patients to determine whether the effect of RNS differs between the median and ulnar nerves.

Results: The decremental responses to RNS were greater in the APB than in the ADM. Reduced CMAP amplitude was negatively correlated with CMAP decrement in median but not in ulnar nerves.

Discussion: The greater CMAP decrement in median nerve was attributed to preferential involvement of the APB in the pathophysiology of ALS or some underlying difference in the biology of the two muscles/nerves. Further investigation may further our understanding of the pathophysiology of ALS.

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P249 RESPIRATORY PREDICTORS OF ALS SURVIVAL IN HOME VENTILATED-COMPLIANT PATIENTS

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Keywords: non-invasive ventilation, survival, predictors

Background: The uncertainty about survival prediction in ALS, is one of the basis for continuous research and effort. Particularly, concerning non-invasive ventilation (NIV) that remains the mainstay strategy in prolonging survival, there is a paucity of trials with rigorous management and optimal parameter settings. We aim to identify potential predictors of survival among ALS patients compliant to NIV.

Methods: Prospective, comparative trial of sixty consecutive ALS patients adherent to NIV (>4h usage/day), who were assigned to two groups according to being alive or dead at February/2012, within a 5-year period of time (from February/2007). G1 (n = 29) included all dead patients who had been compliant to NIV. G2 patients (n = 31) included compliant subjects who were alive in the same period. All patients were followed-up with ALSFRS, oxymetry, respiratory

function testing and blood gases analysis every 3 months, and all data were registered to compare timings to NIV adaptation and usage. Primary outcomes included survival (disease duration from symptoms onset; from diagnosis; and from NIV adaptation). Secondary outcomes included ALSFRS scores; nocturnal pulse oxymetry and parameters data from the BiPAP ventilator. Statistics: groups compared with ANOVA and chi^2 as appropriate, as well as multivariate Cox regression analysis to determine independent predictors of survival.

Results: No clinical or laboratorial differences were observed between groups for any variable at admission (site of disease onset, ALSFRS, RFT and blood gases, or oxymetry) as there were no parameters setting differences at initial NIV trial (pressures; triggers sensitivities; I/E ratio; or back-up breath rate). At the end of the study, statistical differences were observed: mean percentage of oxygen saturation (SpO_2) was higher in G2 ($p = 0.06$), percentage of saturation under 90% was higher in G1 ($p = 0.039$) and mean heart rate was lower in G2 ($p = 0.005$). Disease duration (in days) from symptoms was higher in G2, but not significant. In addition, survival correlated positively with maximal inspiratory pressure (MIP; $p = 0.02$), IPAP ($P = 0.041$) and backup breathing rate (BR; $p = 0.038$). Multivariate Cox regression analysis showed that apart from age, gender and type of disease onset, ALSFRS ($p < 0.002$), SpO_2 ($p = 0.002$), Sat $< 90\%$ ($p = 0.005$), IPAP ($p < 0.0001$), MIP ($p < 0.0001$), periodic pattern ($p < 0.001$) and BR ($p = 0.016$) were associated with increased disease duration and ALSFRS rate of decline. (-2 log-likelihood 103.24; (df) 16; $p = 0.001$).

Conclusions: Determinants of respiratory function (MIP), oxymetry, and parameters setting of NIV equipment are significant predictors of survival. This strengthens the idea of NIV as a potential disease-modifiable treatment strategy as well as the need to fully adapt and follow-up ventilated patients.

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P250 ALS AND VEGF: MODULATION BY NON-INVASIVE VENTILATION

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Keywords: exercise, non-invasive ventilation, VEGF

Introduction: The role of VEGF in the pathogenesis of ALS is a critical point. We have addressed this issue by evaluating VEGF expression in a large population of patients. In addition, we tested the possible role of its modulation by non-invasive ventilation in ALS patients with respiratory failure. In a small group of ALS patients without respiratory symptoms VEGF was quantified before and after exercise.

Methods: We measured plasma level of VEGF in 83 ALS patients, 20 controls and 10 patients with other neuromuscular diseases matched for age. ALS patients were divided into 4 groups: patients presenting with severe respiratory insufficiency before and after non-invasive ventilation (NIV) (G1); stable ALS patients chronically on NIV (G2); patients undergoing a standardized exercise (G3); and stable ALS patients without respiratory impairment.

Results: 30 ALS patients were included in G1, 14 in G2, 12 in G3 and 27 in G4. VEGF levels were similar in controls and in all ALS groups analysed. There was no association between VEGF level and gender, first affected region, age, disease duration and ALS-FRS-R. In general no correlation was found between VEGF level and respiratory function measurements, except for G2 in which VEGF was negatively correlated to forced vital capacity (p 5 0.025). VEGF level increased significantly in G2 after NIV ($p = 0.01$) and in G3 after exercise ($p = 0.02$) but remained stable in the rest of patients, with no significant change in patients tested longitudinally.

Discussion: Overall, in our population plasma levels of VEGF is similar between ALS and controls. However, it seems that non-invasive ventilation increases VEGF expression in ALS patients with marked respiratory impairment. Moreover, exercise has the potential to augment VEGF plasma level in ALS patients. Our results suggest that both non-invasive ventilation and exercise can drive neuroprotective mechanisms in ALS.

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P251 LONGITUDINAL CHANGES OF OUTCOME MEASURES IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Keywords: natural history, biomarker, CAG repeats

Background: Several agents have been shown to prevent or slow disease progression in animal models of spinal and bulbar muscular atrophy (SBMA), although no previous clinical trials showed definite efficacies.

Objectives: To analyze natural history of SBMA with quantitative outcome measures and to establish sensitive and validated disease-specific endpoints for the clinical trials.

Methods: We performed a prospective observation of disease progression over 3 years in 34 genetically confirmed Japanese patients with SBMA by using quantitative outcome measures, including functional and blood parameters.

Results: The baseline evaluation revealed that CAG repeat length in the *androgen receptor* gene correlated not only with the age of onset but also with the timing of substantial changes in activity of daily living. Multiple regression analyses indicated that the serum levels of creatinine are the most useful blood parameter that reflects the severity of motor dysfunction in spinal and bulbar muscular atrophy. In 3-year prospective analyses, a slow but steady progression was affirmed in most of the outcome measures we examined. In the analyses using random coefficient models that summarize

the individual data into a representative line, disease progression was not affected by CAG repeat length or onset age. These models showed large inter-individual variation, which was also independent of the differences of CAG repeat size. Analyses using these models also demonstrated that the subtle neurological deficits at an early or preclinical stage were more likely to be detected by objective motor functional tests such as the 6-minute walk test and grip power or serum creatinine levels than by functional rating scales such as the revised amyotrophic lateral sclerosis functional rating scale or modified Norris scale. Categorization of the clinical phenotypes using factor analysis showed that upper limb function is closely related to bulbar function, but not to lower limb function at baseline, whereas the site of onset had no substantial effects on disease progression.

Discussion and conclusion: These results suggest that SBMA patients show a slow but steady progression of motor dysfunction over time that is independent of CAG repeat length or clinical phenotype, and that objective outcome measures may be used to evaluate disease severity at an early stage of this disease.

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