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SESSION 1 JOINT OPENING SESSION

C1 END OF LIFE DECISIONS: THE PALLIATIVE CARE VIEWPOINT

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Keywords: quality of life, symptom control, patient-centred

Palliative care for patients with neurological disease begins at the time of diagnosis; cure is not an option. Patients are in turmoil, facing their world disintegrating in front of them. Care needs to focus around the physical, improving their environment and controlling symptoms, as well as focusing on the emotional adjustment and on social and spiritual concerns. Patients will be grieving for their lost hopes and fears, for the life that they realise that they will not be able to enjoy and for unfulfilled dreams.

At the same time, the family needs support to come to terms with the difficult situation that they face. Family members are often the key carers, fearful of what lies ahead, not knowing who to call and fearing that they will be abandoned in their hour of greatest need.

Decision making in palliative care has to always weigh up the benefits against the risks and burdens to the patient in the broader context of their social environment as well as in the wider context of any co-morbidity. Improving quality-of-life requires an adjustment of hopes and expectations so that they are more achievable within the reality of disease, simultaneously with an improvement in all aspects of the patient's reality of a lived daily experience on a day-to-day basis.

Children in the family may often bear much of the burden of caring. When the patient is a young parent, children can feel culpable for the disease itself. Children need clear explanations of what is happening and to have their questions answered, knowing that they can ask more questions. Their school needs to be aware of a parent's illness and may need a supportive letter from the clinical team to explain the stresses that the child is under. Many families overlook the profound bereavement that occurs as the child sees a parent's health failing. Children pushed away, excluded from caring, can feel increased isolation and rejection. They often want to be involved in care; in bereavement this can be a source of support as much adjusting will have happened prior to the parent's death.

If the decision is made to cease treatment, such as discontinuing ventilation, the patient must receive full support and care until death occurs. It is the patient's decision to consent to ongoing treatment, or to withdraw that consent. Such decisions need to be fully informed and every effort must be made to help the patient communicate their decision. In the event of the patient lacking capacity to take a decision, the clinician carries responsibility for a 'best interest' decision, taken in the best interest of the patient as an individual person.

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C2 THE WINDING ROAD TO TREATING ALS

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Keywords: disease models, modifying factors, treatment

Progress in the understanding of the biology of amyotrophic lateral sclerosis (ALS) has been significant thanks to a large set of data gathered in genetic, cellular and mouse studies. Several ALS causing genes have been identified and common themes through which they damage motor neurons are beginning to emerge. The cause of sporadic ALS however remains unknown. Therefore, animal models based on the familial forms of ALS remain essential to identify factors that modify motor neuron degeneration in all forms of the disease, and which could represent targets for therapeutic intervention. For this purpose, models based on rodents, fish, flies and worms are being used, all with intrinsic strengths and weaknesses.

In spite of the exciting results obtained using these approaches over the last decade, translation into a therapeutic strategy for ALS patients has proven to be difficult. The reason for this is multifactorial and certainly not only due to the validity of the models used. Confirmation of effects found should keep us from investing in false positive results, while rigorous pharmacodynamic and pharmacokinetic studies should prevent discarding potentially interesting molecules. Collaborations between academic labs with each a specific expertise and between academia and the private sector will increase our chances of being successful.

Although the absence of a therapeutic breakthrough so far is a reality we have to face, numerous accomplishments in ALS are very encouraging, especially compared to other similar fields. Mice that overexpress mutant SOD1, a cause of dominant ALS, develop a late onset, fatal and quantifiable motor neuron disease. The discovery of mutations in unrelated proteins allows testing of compounds independent of the "type" of ALS. Knockdown of a specific mutant protein becomes feasible. New methods for the delivery of compounds are being explored. Trial methodology is well established and remains the topic of constructive criticism. An intense interaction between patients and clinicians has established a network that is able to randomize a large number of patients in a very short time, and at the same time has tremendously increased the quality of care for ALS patients, as is clear from the changing natural history of the disease. The number of research groups and their output exponentially rises. Interest from the private sector in ALS increases rather than fades.

It is very likely that a combination of drugs will be used based on general rules but also on the "type" of ALS and characteristics of the patient. This will require fast and reliable testing in an individual patient. The availability of a biomarker, easily accessible and truly reflecting the evolution of the disease, will be pivotal.

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C5 INTERACTION PARTNERS FOR MISFOLDED SOD1

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Keywords: misfolding, protein interaction, proteomics

Background: Mutations in the free radical scavenging enzyme Superoxide dismutase-1 (SOD1) were linked to ALS in 1993 and for the last 15 years there has been intense research regarding the role of SOD1 in ALS. Still the precise mechanism by which mutant SOD1 is toxic to motor neurons is unknown. One of the dominating hypotheses about SOD1 toxicity concerns protein misfolding. A role for SOD1 misfolding in ALS is indicated by several lines of evidence. At autopsy, large intraneuronal inclusions that stain for SOD1 are found in patients with SOD1 mutations, rodents that overexpress mutant SOD1 generate similar inclusions at endstage and soluble misfolded SOD1 is enriched in spinal cords of different transgenic ALS model mice. Also, studies on recombinant SOD1 have shown that mutations in SOD1 destabilize the precursor monomers and weaken the dimer interface.

Objectives: When SOD1 misfolds, internal structures normally hidden in the hydrophobic core become exposed. These sticky parts of the molecule might form non-native interactions and in this way be cytotoxic to motor neurons. Here we set out to identify novel interaction partners for misfolded SOD1.

Methods: We have created a set of anti-peptide antibodies that are highly specific for misfolded SOD1. For this study, an antibody directed towards the C-terminal of SOD1 was used since this part of the molecule is missing in ALS-linked truncation mutants. Thus the C-terminal antibody will not interfere with any interaction relevant for ALS pathogenesis. The antibody was coupled to Sepharose and used to immunocapture misfolded SOD1 in spinal cord extracts from transgenic mice. The misfolded SOD1 together with interacting proteins is then released by addition of the peptide used to raise the antibody. With this paradigm, only interaction partners to the supposedly toxic misfolded SOD1 are isolated. Interacting proteins are identified by proteomic techniques including 2D-PAGE and mass spectrometry, but also in a candidate approach by western immunoblots to verify or rule out interesting conceivable binding partners.

Results: Misfolded SOD1 interacts with several heat shock proteins and chaperones including Hsc70 and Grp78 and also to cytoskeleton proteins like actin and actin related proteins.

Discussion: Identification of binding partners for misfolded SOD1 might lead to new hypothesis about the cytotoxic mechanism exerted by mutant SOD1s.

C6 TEMPLATE-DIRECTED MISFOLDING OF SOD1 *IN VITRO*: A MODEL FOR ALS PROPAGATION?

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Background: Protein misfolding diseases probably comprise several different classes of pathogenesis. Prion diseases are thought to propagate through template-directed misfolding (TDM) of the cellular prion protein PrP^{C} by the disease-misfolded prion protein PrP^{Sc}. Recent discoveries suggested that a TDM mechanism might participate in the pathogenesis of Alzheimer's disease (AD), and seeded aggregation *in vitro* has been observed for many proteins and peptides implicated in AD, PD, and ALS. Mutations in SOD1 are associated with ~20% of familial amyotrophic lateral sclerosis (ALS). When mutated and/or oxidized, superoxide dismutase 1 (SOD1) is subject to misfolding and aggregation, and is efficiently exported from the cell. We have hypothesized that ALS propagates via the template-directed misfolding mechanism similar to that thought to underlie the prion diseases.

Objectives: To establish whether mutant misfolded SOD1 can induce misfolding and aggregation of natively structured wild-type SOD1 intracellularly, and if propagation of SOD1 misfolding can be transmitted from cell-to-cell.

Methods: We exploited G127X, a natural familial ALS frameshift mutation, in transfection and immunoprecipitation (IP)/immunocytochemistry (ICC) paradigms in which the mutant, wild-type (wt) and misfolded wtSOD1 species could be unambiguously identified by antibodies specifically directed against these molecular species, including monoclonal antibodies directed against disease-specific epitopes of misfolded SOD1. Characterization studies of misfolded/aggregated SOD1 species were also obtained by solubility in non-denaturing detergents, and protease resistance using proteinase K (PK).

Results: wtSOD1 misfolding-specific antibody immunoreactivity is induced by contact with G127X mutant SOD1 as determined by IP and ICC. Misfolded wtSOD1 acquires PK sensitivity, but not detergent insolubility. Misfolded SOD1 (mutant and wt) acquire non-native inter-chain disulfide bonds, although the induction of misfolding of wtSOD1 is preserved with G127X mutants in which all remaining cysteines were mutated to serine residues by *in vitro* mutagenesis. G127X SOD1-induced misfolding of wtSOD1 is not observed in mouse cell lines. We have detected misfolded mutant and wtSOD1 in the media of G127X SOD1-transfected cells; non-transfected cells incubated in the presence of this medium can induce SOD1 misfolding intracellularly. Incubation of G127X SOD1-transfected cells with poly-specific SOD1 antibodies reduces wtSOD1 misfolding *in vitro*.

Discussion: Misfolded SOD1 can induce wtSOD1 misfolding by non-covalent interaction in human neural and mesenchymal cell lines. G127X-induced wtSOD1 acquires PK sensitivity consistent with conformational loosening. Non-native disulfide bonds are a consequence (not a cause) of wtSOD1 misfolding, but may "lock in" wtSOD1 misfolding, and/or stabilize G127X interactions for co-immunoprecipitation. Misfolding of wtSOD1 respects a "species barrier" between human and mouse proteins. Furthermore, misfolding of SOD1 propagates in cell culture by intercellular exportation and uptake of antibody-accessible misfolded molecular species.

Conclusion: Misfolded SOD1 can engage in template-directed misfolding, a possible explanation for the prion-like propagation of ALS in the neuroaxis.

C7 KINETIC ANALYSIS OF SOD1 MISFOLDING RESULTING FROM OXIDATIVE DAMAGE: POTENTIAL RELEVANCE TO FAMILIAL AND SPORADIC ALS

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Background: Mutations in the gene encoding the Cu, Zn superoxide dismutase (SOD1) are known to cause approximately 20–25% of familial ALS cases, as well as a considerable number of apparently sporadic ALS cases (1,2). Mutation is believed to promote SOD1's entry into a cytotoxic misfolded conformation (3). It is possible that oxidative damage to the protein or other stresses may also trigger SOD1 misfolding, suggesting a potential mechanism by which wild-type SOD1 could cause sporadic ALS (4). We previously developed kinetic assays for examining the release of SOD1's bound metals, unfolding of the SOD1 beta-barrel, and dissociation of the SOD1 dimer (5). Using these assays, we were able to elucidate the unfolding mechanism for wild-type SOD1 denatured in guanidine hydrochloride (5).

Objectives: This study aims to apply the previously developed techniques to elucidate the mechanism of SOD1 misfolding triggered by oxidative damage.

Methods: SOD1 was treated with millimolar hydrogen peroxide (H_2O_2) in the presence of buffer and 100 μM 4-(2-pyridylazo)resorcinol (PAR). Kinetic measurements of PAR absorbance allowed determination of rates of copper and zinc release resulting from H_2O_2-induced damage. Kinetic tryptophan fluorescence measurements were used to examine beta-barrel conformational changes. Data were analyzed via model-free Laplace methods using a custom algorithm, and were also fit to various sequential release models by least-squares methods.

Results: H_2O_2 appeared to induce damage selectively at the SOD1 active site, inducing release of both copper and zinc. No change in tryptophan fluorescence was observed, suggesting that global structural rearrangements do not result from H_2O_2 treatment. Both metals were released via three-state sequential mechanisms, each step involving one molecule of hydrogen peroxide, suggesting that two metal-binding histidine residues must be oxidatively modified to trigger release of a metal atom. Copper release was total but zinc release was not, consistent with a model in which the catalytic copper's presence is necessary to convert H_2O_2 to superoxide (O_2^-), which then damages histidine residues.

Discussion and Conclusions: SOD1's role as a scavenger of free radical species comes with the occupational hazard of damage caused by those species. The preliminary findings presented here suggest that this damage can result in partial or total loss of copper and zinc without global destabilization of the protein, resulting in native-like metal-deficient/misfolded SOD1 species. These are candidates for cytotoxic species that may play a causal role in ALS.

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C8 MODIFICATIONS OF SUPEROXIDE DISMUTASE IN HUMAN ERYTHROCYTES: A POSSIBLE ROLE IN ALS

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Keywords: biophysics, molecular modeling, superoxide dismutase

Background: Over 100 mutations in Cu/Zn-superoxide dismutase (SOD1) result in familial amyotrophic lateral sclerosis. Dimer dissociation is the first step in SOD1 aggregation, and studies suggest nearly every amino acid residue in SOD1 is dynamically connected to the dimer interface. Post-translational modifications of SOD1 residues might be expected to have similar effects to mutations, but few modifications have been identified.

Objectives: Our objective was to test the hypothesis that SOD1 is modified in humans, determine these modifications, and understand their role in promoting SOD1 dissociation, which is the first step in the SOD1 aggregation pathway.

Methods: We used size-exclusion chromatography for separation of modified SOD1 species from the unmodified ones. We used a combination of "bottom-up" and "top-down" mass spectrometry approaches to search and to identify the modifications. To measure Kd we used SOD1 activity as an assay: we tested for a reduction in the rate of 6-hydroxydopamine.

Results: Here we show, using SOD1 isolated from human erythrocytes, that human SOD1 is phosphorylated at threonine 2 and glutathionylated at cysteine 111. A second SOD1 phosphorylation was observed and mapped to either Thr-58 or Ser-59. Cysteine 111 glutathionylation promotes SOD1 monomer formation, a necessary initiating step in SOD1 aggregation, by causing a 2-fold increase in the Kd. This change in the dimer stability is expected to result in a 67% increase in monomer concentration, 315nm rather than 212nm at physiological SOD1 concentrations.

Discussion: The link between SOD1 mutations, protein aggregation and FALS is not fully understood, but there are multiple reports showing that dimer dissociation is an early event during SOD1 aggregation. Our finding that modifications can facilitate SOD1 dimer dissociation suggests a possible link between the normal characteristics of SOD1 and its role in FALS. Although relatively modest, a 2-fold increase in Kd resulting from SOD1 modification translates to nearly a 70% increase in SOD1 monomer concentration. Because nucleation of SOD1 aggregation is dependent on at least the square of the monomer concentration, we expect a 70% increase in monomer concentration to have a marked effect on the nucleation of SOD1 aggregates. For example, the formation of a hypothetical nucleus made up of 3 glutathionylated SOD1 monomers becomes roughly 5 times more likely and a nucleus of 6 monomers 24 times more likely relative to unmodified SOD1.

Conclusions: Because protein glutathionylation is associated with redox regulation, our finding that glutathionylation

promotes SOD1 monomer formation supports a model in which increased oxidative stress promotes SOD1 aggregation.

C9 PROTEIN DISULPHIDE ISOMERASE IS S-NITROSYLATED IN ALS

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Keywords: protein disulphide isomerase, ER stress, S-nitrosylation

Background: Endoplasmic reticulum (ER) stress is a key feature of ALS pathology, occurring early in disease in transgenic mutant SOD1 ALS models and also in spinal cords of human patients. ER stress occurs when misfolded proteins accumulate in the ER, triggering a homeostatic mechanism known as the unfolded protein response (UPR). Activation of the UPR causes up-regulation of chaperones such as protein disulphide isomerase (PDI), which modulates disulphide bond formation. However, prolonged activation of the UPR leads to apoptotic signaling. In ALS, PDI is highly up-regulated throughout disease course and in mutant SOD1 mice prior to the onset of symptoms. Over-expression of PDI is protective against mutant SOD1 inclusion formation and toxicity, suggesting that protein aggregation and ER stress are involved in ALS pathogenesis. Recently, S-nitrosylated PDI (SNO-PDI) was detected in brains of Alzheimer's and Parkinson's disease patients, providing a further link between PDI function and protein misfolding in neurodegeneration. S-nitrosylation of PDI involves aberrant post-translational modification whereby nitrosative stress triggers the transfer of nitric oxide groups to critical active site cysteine residues, resulting in functional inactivation of the enzyme.

Objectives: The aim of this study was to determine whether or not PDI modification by S-nitrosylation occurs in spinal cord of human ALS patients and transgenic mutant SOD1 mice.

Methods: Lumbar spinal cord tissues from ALS patients and controls without evidence of neurological disease were extracted at post-mortem. Spinal cord tissues from $SOD1^{G93A}$ transgenic mice, along with non-transgenic littermate controls, were also used. SNO-PDI was detected using a biotin switch assay in which S-nitrosylated cysteine residues were specifically biotinylated, followed by precipitation of samples with streptavidin-agarose and immunoblotting. Levels of SNO-PDI were normalised to the amount of total PDI in each sample by densitometry.

Results: SNO-PDI was highly increased in lumbar spinal cord tissue of ALS patients compared to controls, even when normalised to total PDI to account for the significantly increased total PDI levels in ALS patients. S-nitrosylated PDI was also detected in $SOD1^{G93A}$ mouse spinal cords but not in non-transgenic littermate controls.

Discussion and Conclusions: S-nitrosylation of critical cysteine residues has previously been demonstrated to prevent the normal protective function of PDI. Although there is a large up-regulation of PDI in ALS, the detection of SNO-PDI in spinal cords of ALS patients and $SOD1^{G93A}$ mice in this study suggests that inactivation of PDI could contribute to disease pathogenesis. Therapeutics targeting PDI or ER stress may therefore be beneficial in ALS.

SESSION 2A PROTEINOPATHIES

C3 PROTEIN AGGREGATES IN NEURODEGENERATIVE DISEASES

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Keywords: neurodegenerative diseases, protein aggregates, tau alpha-synuclein

Several neurodegenerative diseases of the nervous system are characterised by the presence of extracellular and/or intracellular protein aggregates. Deposits of ß-amyloid and microtubule-associated protein tau are characteristic of Alzheimer's diseases (AD) but tau deposits can also be found in diseases such as progressive supranuclear palsy, corticobasal degeneration, Pick's diseases/frontotemporal dementia (FTLD-T) that are now known as "tauopathies" (1). More recently insoluble tau aggregates have been also identified in progressive forms of multiple sclerosis (2). The major component of the inclusions present in Parkinson's diseases (PD), dementia with Lewy bodies and multiple system atrophy is instead alpha-synuclein and these diseases are now named "alpha-synucleinopathies" (3). Forms of FTLD characterized by the presence of ubiquitin inclusions (FTLD-U) can have as major component of the aggregates TDP-43 that is also present in protein deposits in motor neuron diseases (MND) (4). The identification of similar protein aggregates in FTLD and MND has shown that these disorders are at the extreme ends of a disease spectrum containing FTLD-MND. Interestingly, while it was believed that protein aggregates were disease specific it is now clear that in some cases different proteins can aggregate in the same disease, for example in AD besides ß-amyloid and tau also alpha-synuclein and TDP-43 deposits can be present. Furthermore, epidemiological and genetic studies have shown that even if protein deposits do not coexist proteins can still interact as in PD where no tau deposits are found with alpha-synuclein inclusions but a certain tau gene haplotype increases susceptibility for the disease (5). The relevance of protein aggregates for disease pathogenesis has become clear with the discovery that mutations in the genes encoding these proteins such as amyloid precursor protein, tau, alpha-synuclein, TDP-43 and FUS cause rare familial forms of the diseases (6–9). The findings in familial cases indicate that also in sporadic diseases a direct link exists between the aggregated protein and disease pathogenesis. Understanding how and why these aggregates form and cause cell death will lead to the design of mechanism-based therapies for the cure of neurodegenerative diseases.

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C4 ROLE OF TDP-43 AND FUS IN DISEASE PATHOGENESIS

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Keywords: TDP-43, FUS, pathogenesis

In 2006, the transactive response (TAR) DNA binding protein with Mr 43 kD (TDP-43) was identified as the pathological protein in a common subtype of frontotemporal lobar degeneration (now referred to as FTLD-TDP) and amyotrophic lateral sclerosis (ALS), thereby providing strong evidence that FTLD, ALS with dementia and classical ALS are all part of a clinicopathological spectrum of disease. Subsequently, mutations in TARDBP, the gene encoding TDP-43, were identified in familial ALS and FTLD, emphasizing that TDP-43 dysfunction is directly involved in disease pathogenesis and neurodegenerative processes in these conditions. TDP-43 is a DNA/RNA-binding protein which becomes abnormally phosphorylated, cleaved and ubiquitinated in disease process. The development of novel antibodies against disease-specific modifications of TDP-43, such as phopshorylation-specific antibodies, or epitope specific antibodies are not only valuable tools for diagnostic purposes, but have already improved our knowledge on the TDP-species composition in abnormal inclusions. Thus, cortical inclusions are selectively enriched for hyperphosphorylated C-terminal fragments over full-length TDP-43 compared to spinal cord inclusions thereby suggesting that TDP-43 is differentially processed in brain versus spinal cord. It is expected that elucidating the functional consequences of disease-associated TARDBP mutations, e. g. by generation of transgenic mouse models, will provide important insights into the disease mechanisms underlying ALS and FTLD-TDP.

Only recently, missense mutations in the gene encoding the fused in sarcoma (FUS) protein (also known as translated in liposarcoma, TLS), another DNA/RNA binding protein with striking structural and functional similarities to TDP-43, were identified as a cause of familial ALS. Because of the recognized overlap between ALS and FTLD we investigated the possible role of FUS in FTLD cases. While no FUS pathology was identified in cases of FTLD with TDP-43 or tau pathology, or TDP-43-positive ALS, we were able to demonstrate FUS as the pathological protein in neuronal cytoplasmic and intranuclear inclusions in cases of tau/TDP-43-negative FTLD, a FTLD subtype accounting for up to 10% of FTLD cases. Immunoblot analysis confirmed the presence of insoluble FUS in post-mortem brain tissue from these cases. No mutations in the FUS gene were identified in any of our FTLD patients with FUS pathology. These findings suggest that FUS is also the pathological protein in a significant subgroup of sporadic FTLD thereby reinforcing the concept that FTLD and ALS are closely related conditions. In summary, the identification of two DNA/RNA binding proteins, TDP-43 and FUS, as pathological proteins in ALS and FTLD, implies alterations in RNA processing as a key event in the pathogenesis of these diseases.

SESSION 2B TRANSLATING EVIDENCE INTO PRACTICE

C10 PERFORMANCE MEASURES AND ACCOUNTABILITY

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The quality and safety of today's medical care are quite variable and there is growing concern about runaway health care costs. Studies have demonstrated poor adherence to guidelines, indefensible variations in care, high cost and waste. As a result, there is growing distrust of the medical profession and calls for greater accountability by consumers, payers and regulators. A variety of methods are used to achieve accountability including competition, transparency, differential payment, accreditation, regulation and litigation. All of these approaches rely on performance measures even though measuring quality of care is in its infancy. Today's emphasis on information technology to assure accountability has created high societal expectations that can only be met if greater emphasis and resources are applied to measure quality of care.

Common measures of health care quality include: (1) how health care is organized (structure); (2) what is done (process); and (3) what happens to the patient (outcome). Because outcomes are the most difficult to obtain, many systems rely heavily on structure and process measures to hold physicians accountable. Successful approaches require system thinking, a culture of process improvement, and the development of performance data that truly reflect cost-effective patient care. Barriers that have to be overcome include: insufficient evidence, the cost of information technology, a perceived threat to physician autonomy, a traditional silo approach to health care delivery, and competing priorities.

Increasingly, academic medicine must support fundamental research in quality, safety and efficiency. Successful models for quality assessment and improvement will be presented and include creating and implementing data registries, developing and promoting cross department collaboration and fostering a no-blame culture of accountability. Future medical practice must extend beyond the quest for medical innovation to incorporate systems and contexts for using medical discoveries cost-effectively.

C11 AAN PRACTICE PARAMETER UPDATE: THE CARE OF THE PATIENT WITH AMYOTROPHIC LATERAL SCLEROSIS (AN EVIDENCE-BASED REVIEW)

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Keywords: evidence-based medicine, management of ALS, practice parameters

Background: The American Academy of Neurology (AAN) issued an evidence-based report on managing patients with amyotrophic lateral sclerosis (ALS) in 1999.

Objective: To systematically review evidence bearing on the management of patients with ALS and update the 1999 AAN practice parameter.

Methods: The authors completed a systematic literature review from 1998 to 2008. Topics included breaking the news, symptom management, slowing disease progression, nutrition, respiratory management, palliative care, cognitive and behavioral impairment, multidisciplinary clinics, and communication for patients with ALS.

Results: The authors identified 10 Class I studies, 13 Class II studies, and 73 Class III studies in ALS. More studies are clearly needed to examine the best tests of respiratory function in ALS, the optimal time for starting PEG, the impact of PEG on quality of life and survival, the effect of vitamins and supplements, symptomatic therapies and palliative care. The following recommendations are made based on the studies analyzed: Riluzole should be offered to slow disease progression (Level A). Percutaneous endoscopic gastrostomy (PEG) should be considered to stabilize weight and to prolong survival (Level B). Noninvasive ventilation (NIV) should be considered to treat respiratory insufficiency in order to lengthen survival (Level B), and may be considered to slow the decline of forced vital capacity (Level C) and improve quality of life (Level C). Early initiation of NIV may increase compliance (Level C), and insufflation/exsufflation may be considered to help clear secretions (Level C). Multidisciplinary clinic referral should be considered to optimize health care delivery and prolong survival (Level B) and may be considered to enhance quality of life (Level C). For the treatment of refractory sialorrhea, botulinum toxin B should be considered (Level B) and low-dose radiation therapy to the salivary glands may be considered (Level C). For treatment of pseudobulbar affect, the combination therapy of dextro-

methorphan with quinidine should be considered, though side effects are not uncommon and the treatment is currently not approved by the U.S. Food and Drug Administration (Level B). For patients who develop fatigue while taking riluzole, withholding the drug may be considered (Level C). Because many patients with ALS demonstrate cognitive impairment, which in some cases meets criteria for dementia, screening for cognitive and behavioral impairment should be considered in patients with ALS (Level B). Other management strategies all lack strong evidence.

Discussion: There are many treatments available for patients with ALS that can alleviate suffering. NIV, PEG, riluzole, and multidisciplinary clinics are the most important and have the best evidence.

Conclusions: More high-quality, controlled studies are needed to guide management and to assess outcomes in patients with ALS.

C12 MEDICATION IN THE LAST DAYS OF LIFE OF MND/ALS – A STUDY FROM SPECIALIST PALLIATIVE CARE PROVIDERS IN THE UK

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Keywords: opioids, palliative care, end of life

Background: Specialist palliative care providers (SPC) are often involved in the care of people with ALS/MND in the UK, particularly in the management of symptoms at the end of life. Despite this experience in the use of medication at the end of life, many patients and their families and professionals fear the last days of life with ALS/MND and the use of medication at this time.

Objectives: This study aimed to show the medication used in the last 72 hours of life within six specialist palliative care units in the UK and Ireland.

Methods: Six SPC units provided details of the last 10 patients who had died under their care. Patient information was collected together with the details of medication used in the last 72 hours before death.

Results: 60 patient records were audited −63% male and 37% female with a mean age of 67 years. The mean time from first symptom to death was 32 months. The majority of patients received medication in the last 72 hours of life, primarily:

- Morphine – 23 patients in the last 24 hours, commonly (38%) by subcutaneous infusion with a mean dose of 80mg (oral equivalent) over 24 hours
- Midazolam – 35 patients, commonly by subcutaneous infusion with a mean dose of 31mg/24 hours
- Anticholinergic medication - as glycopyrronium bromide or hyoscine hydrobromide - 35 patients

All patients were reported as dying peacefully, without distress.

Discussion and Conclusion: This study showed that medication is commonly given within SPC units for the management of symptoms at the end of life. The doses used are similar to those in other studies and in surveys of cancer patients - for ALS/MND the studies showed a mean oral equivalent dose of morphine of 98mg/24hours (1) and 90mg/24 hours (2), and for cancer patients a mean dose of 166mg/24 hours (3).
The results show that professionals can feel secure in the administration of medication at the end of life and the doses used are not large and similar or less than for other terminal care groups. Patient and families can also be reassured that with good symptom management and the best use of medication dying form ALS/MND is peaceful.

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C13 FACTORS UNDERLYING END OF LIFE-DECISIONS IN ALS PATIENTS

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Keywords: end-of-life decision, depression, cognitive processes

Background: Over the course of the disease, ALS patients have to make decisions with regards to life-sustaining treatment (non-invasive ventilation, NIV; percutaneous endoscopic gastrostomy, PEG; invasive ventilation, IV). Depression and quality of life were found to be independent of the progression of the disease but to be predictors of the wish to die and physician-assisted suicide (PAS). Data on the processes underlying these end-of life decisions are sparse.

Objectives: Our investigation aimed to identify factors underlying the end-of-life decision by a longitudinal approach. The course of depression, subjective quality of life, attitudes toward life sustaining treatment and hastened death, as well as the development of the decisional process itself were investigated.

Methods: Patients with definite ALS and no cognitive impairment were eligible to participate in the study. The time between interviews was 6 months. Depression, subjective quality of life and attitudes toward hastened death were assessed by standardized instruments. Attitudes and other cognitive factors regarding the end-of-life decision were assessed by the Life Sustaining Treatment Questionnaire (Häcker, 2008, unpublished).

Results: T1: The sample consisted of 61 patients. Depression was neither related to demographic or disease related variables (time since diagnosis, bulbar symptoms, pain, ventilatory status) nor associated with the wish to die. Level of depression was related to quality of life ($r = -0.513$), fear of death ($r = 0.324$), and the wish for legalizing PAS ($r = 0.542$). Quality of life was highest in IV-patients and was not associated with disease progression or bulbar symptoms. Low quality of life was related to stronger approval of the legalisation of PAS ($r = -0.438$) and a stronger wish to die. About half of the sample had not made a decision regarding life sustaining treatment yet. Individuals reporting a positive decision toward life sustaining treatment and those who had not made a decision yet, reported a low wish to die. The highest wish to die was

found in patients who decided against life sustaining treatment.
T2: Present results suggest that depression, quality of life and the wish to die do not change over time. Only the fear of death decreased significantly.

Discussion and Conclusion: Most strikingly, attitudes toward life sustaining/shortening treatment might not be related to the disease itself. Rather, they seem to be mediated by psychological processes that could be subject to intervention. During all stages of the disease, a high quality of life and low levels of depression are possible. The results can contribute to the general debate over the legalisation of PAS. The focus of this debate should lie on the necessity to ensure every possible support to ALS patients to improve their well being towards the end-stage of the disease.

C14 HOW PATIENTS WITH ALS, MAKING ADVANCED DECISIONS, CAN INFLUENCE OUTCOMES IN WHERE THEIR END OF LIFE CARE IS RECEIVED

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Keywords: preferred priorities of care (PPC), end of life care, place of death

Objectives: The Preferred Priorities of Care document (PPC) gives the terminally ill patient an opportunity to think, talk about and write down preferences and priorities for end of life care. Our aim was to establish if patients had specified a choice of where their care would be received at their end of life, that this had been achieved and if not what had happened to prevent this.

Methods: Comparisons were made between three groups of patients with ALS, those who completed a PPC, those who had had discussions with the MND specialist nurse with regard to their PPC but had not formally recorded their wishes and those who did not wish or had not had the opportunity to complete a PPC. Data recorded included their preferred place of terminal care.

Results: Of the 44 people who chose to complete preferred priorities of care, 39 stated they would prefer to die at home. Twenty-five of these achieved their death at home, three in a hospice, nine in hospital, two in nursing homes. Two patients wished to die in a hospice as their second choice and this was achieved. Two chose to die in hospital and one in a nursing home.
Of the 45 patients who did not complete a PPC eighteen died at home, sixteen in hospital, eight in a nursing home and two in a hospice. Fourteen patients had had discussions about PPC but had not made decisions on place of terminal care. Six of these died in hospital, five at home, two in a nursing home and one in a hospice.
The main reasons for not achieving their desired PPC included carers being unable to cope and sudden change in medical condition.

Discussion: It is suggested that in the UK between about 80–90% of those with a terminal illness expressed a preference for death at home but 60% die in hospital. The results of our audit suggest that patients have a higher chance of achieving their preferred place of end of life care if their wishes have been recorded on a PPC. Further investigations should be made to establish causes of breakdown in care leading to hospital admission in the last few days or hours of life and how these admissions can be prevented.

C15 CARING FOR THE CAREGIVER PART 1: USING EVIDENCE BASED PRACTICE TO DESIGN INTERVENTIONS TO SUPPORT ALS CAREGIVERS

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Keywords: caregiving, quality of life, evidence based practice

Background: Information on how to support caregivers of ALS patients is sparse. An Evidenced Based Practice (EBP) approach was used to identify potential interventions to support caregivers. EBP is the process of systematically searching for the best available evidence to support a clinical decision or clinical intervention.

Objectives: To report on the process by which we used EBP to develop a questionnaire which can be used to design interventions to support ALS caregivers.

Methods: A multidisciplinary team used EBP to gather and analyze available information on what can be done to support ALS caregivers. Information on caregiving was obtained from three sources: 1) published literature; 2) expert opinions; 3) caregiver focus groups. The information was reviewed for potential intervention development.

Results: Published literature: 70 relevant articles were retrieved, 21 of which were applicable to intervention development: Level 1 (systematic review or multicenter controlled studies) – 5 articles; Level II (single-center controlled studies) – 4 articles; Level III (case-control studies) – 9 articles; Level IV (qualitative reviews or studies) – 3 articles. The study population in the articles included 393 ALS caregivers, 4154 Alzheimer's caregivers, and 2062 other caregivers. The literature revealed the following: early interventions are most effective; high risk-factors and protective functions for caregivers can be identified; interventions should be multifocal; strategies exist to decrease caregiver perceptions of burden; extra supports aid well-being. Expert opinions generated by clinicians from an interdisciplinary ALS clinic supplemented the literature: educate about all aspects of the disease at 3 month intervals; identify sources of stress and coping; identify available support systems; caregiving experiences differ depending on the relationship to the patient; caregivers need encouragement to call the ALS team for assistance. Caregiver focus groups reinforced services that are helpful (hospice; repeating information about services; emotional/social supports from friends/community) and identified areas in which more attention is needed (finding good in-home care; providing information and options about equipment; removing physical signs after the loved one dies). The three sources of information were reviewed by the EBP team, who determined that the design and administration of a Caregiver Assessment Form in ALS clinic would potentially be a useful intervention. The assessment form includes demographics, assessment for caregiver risks (concern with tasks, health and well-being, stress), and protective functions (optimism, confidence, spirituality).

Discussion and Conclusions: EBP can be used to foster collaboration between clinicians and researchers to design and construct an instrument for the assessment of ALS caregivers. Such a tool can form the basis for interventions to maximize the quality of life of caregivers of patients with ALS.

SESSION 3A BIOMARKERS

C16 DISCOVERY AND VERIFICATION OF PROTEIN BASED BIOMARKERS FOR ALS

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Keywords: biomarkers, proteomics, diagnostics

Background: Rapid diagnostic tests for motor neuron disease are not currently available but would assist neurologists in making a clinical diagnosis and more rapidly initiate proper patient treatments to extend quality of life and/or initiate patients into clinical trials at early stages of disease progression. Many groups have discovered candidate biomarkers for ALS, though most have utilized small numbers of test subjects and few proper disease mimics. Continued verification and follow-up validation studies are lacking for all candidate biomarkers and therefore represent an important next step in the ultimate translation of biomarkers from the bench to the clinic.

Objectives: Our goal was to further discover and verify candidate protein based biomarkers for motor neuron disease using mass spectrometry based proteomics and antibody based methodologies using a large cohort of samples.

Methods: Cerebrospinal fluid (CSF) from 85 patients with sporadic ALS, 15 with familial ALS, 18 with multiple sclerosis, 53 with Alzheimer's disease, 29 other disease control, and 41 healthy control subjects was examined by Surface Enhanced Laser Dissociation/Ionization Time-of-Flight mass spectrometry (SELDI-TOF-MS). Mass spectral data were analyzed by multiple computer based algorithms to identify potential biomarker peaks. The mass peaks with high predictive value were validated by enzyme-linked immunosorbent assay (ELISA).

Results: A total of 68 mass spectral peaks were found to be differently expressed between the ALS and healthy control groups. Mass peaks for cystatin C and transthyretin were reduced in ALS patients, whereas mass peaks for a post-translational modified form of transthyretin and C-reactive protein (CRP) were increased in ALS. CRP levels were 5.84 ± 1.01 mg/L for controls and 11.24 ± 1.52 mg/L for ALS subjects as measured by ELISA. Increased levels of free hemoglobin were observed in 56% of ALS patients. The total level of cystatin C correlated to ALS patient survival.

Conclusions: This is the largest ALS biomarker discovery and verification study performed to date. Our study validated our prior mass spectrometry results demonstrating decreased levels of cystatin C and increased levels of specific modified forms of transthyretin in the CSF of ALS patients. CRP levels are increased in the CSF of ALS patients, and cystatin C levels in the CSF correlate to survival of sporadic ALS patients with limb onset disease. We also verified other previously published candidate biomarkers in our study. Our proposed biomarker panel predicted ALS with a high level of specificity (94%) and 82% overall accuracy.

C17 VALIDATION OF A CSF BIOMARKER PANEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: CSF, biomarker, cytokines

Objective: To determine biomarkers associated with ALS in CSF samples obtained from multiple clinics.

Background: Multiple studies have sought biomarkers associated with ALS using a variety of techniques and control groups for comparison. We have previously reported a panel of CSF biomarkers distinguishing ALS patients from a group of neurological disease control subjects. All subjects included in our previous study were evaluated at the ALS Clinic at Penn State Milton *S.* Hershey Medical Center. The current study expands upon our earlier study to include samples collected at other clinics through multiple studies.

Methods: CSF samples were collected through the Northeast Amyotrophic Lateral Sclerosis (NEALS) Consortium. In total, 30 CSF samples were obtained from ALS patients and 30 CSF samples were obtained from healthy control subjects. Subjects were approximately matched for age, ethnicity and sex. We performed multiplex analysis on the CSF samples using the Bio-Plex Human 27-plex panel of cytokines and growth factors (Bio-Rad, Hercules, CA).

Results: The two comparison groups were approximately matched for age (median: 54.0 (ALS) vs. 40.0 (control) years, $p = 0.07$), sex (Chi-square $= 1.11$, $p = 0.29$), and ethnicity (Chi-square $= 2.50$, $p = 0.47$). Similar to the preliminary study, ALS patients demonstrated significantly elevated CSF levels of IL-6 (median: 6.07 vs. 4.58 pg/mL, $p < 0.01$), MCP-1 (218.9 vs. 140.6 pg/mL, $p < 0.001$), and VEGF (29.78 vs. 20.46 pg/mL, $p < 0.05$) compared to the healthy control group, and IFN-γ (12.53 vs. 19.13 pg/mL, $p < 0.05$) was significantly decreased in the ALS patient group. In addition, ALS patients had elevated CSF levels of IL-1 receptor antagonist (9.81 vs. 6.98 pg/mL, $p < 0.05$) and IL-8 (25.19 vs. 19.02 pg/mL, $p < 0.001$), and decreased levels of platelet-derived growth factor (6.14 vs. 10.60 pg/mL, $p < 0.05$), and IL-9 (26.20 vs. 34.11 pg/mL, $p < 0.01$). Analysis revealed a negative correlation between duration of symptoms and MCP-1 ($r = -0.515$, $p < 0.01$).

Discussions and Conclusions: In general agreement with our previous study comparing ALS patients with a group of neurological disease control patients, a number of biomarkers are associated with ALS suggesting inflammatory changes within the CNS. These biomarkers may have utility in allowing earlier diagnosis of ALS, elucidating disease pathogenesis, monitoring disease progress, and determining disease prognosis.

C18 PROTEIN DISULPHIDE ISOMERASE IN CSF - A NOVEL THERAPEUTIC AND/OR BIOMARKER FOR ALS?

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Keywords: biomarker, protein disulphide isomerase, ER stress

Background: Protein Disulphide Isomerase (PDI) is responsible for the formation and isomerisation of native protein disulphide bonds. Mutant SOD1 forms high molecular weight aggregates containing aberrant, non-native disulfide bonds, which are linked to neuronal toxicity and disease progression. Hence, modulation of disulphide bond formation could be important in pathogenesis. We showed recently that PDI: (a) was upregulated in motor neurons from $SOD1^{G93A}$ mice and human patient tissues; (b) was located within cytoplasmic inclusions of motor neurons; and (c) prevented the aggregation of SOD1 *in vitro*. These results suggest that PDI is a novel therapeutic target for ALS and because a good biomarker should be close to the fundamental pathology, possibly also an effective biomarker. At present there is no biochemical diagnostic test for ALS, so a biomarker that could detect early disease or could predict the onset of symptoms and burden of disease would be an excellent diagnostic tool. CSF is an excellent source of biomarkers due to its proximity to brain parenchyma.

Objective: To determine if PDI in CSF has potential as a biomarker for ALS.

Methods: CSF from $SOD1^{G93A}$ and non-transgenic control rats was collected at p60, p90 and p120. CSF was obtained from 7 human patients with early disease, 8 patients with late stage disease, and from 5 controls without neurological disease. Western blotting was performed to detect the expression level of PDI in CSF.

Results: We detected markedly elevated levels of PDI in CSF from both $SOD1^{G93A}$ rats and human patients compared to controls (over 5 fold; $p < 0.01$). Furthermore, there was a two fold elevation of PDI in CSF taken from patients at the diagnosis of sporadic MND ($p < 0.001$). However, by late disease (taken as close as possible to the date of death), this elevation of PDI increased to three-fold ($p < 0.01$) suggesting that PDI increases with disease progression. Importantly, PDI was elevated in the CSF (4 fold, $p < 0.01$) in animals 30 days prior to the onset of signs.

Discussion and Conclusions: PDI was greatly elevated in the pre-symptomatic phase of disease in the CSF of $SOD1^{G93A}$ rats. In human patient CSF, its expression levels increased with disease course. These data suggest that PDI has potential as novel biomarker for ALS.

C19 NITRATED PROTEINS IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF ALS PATIENTS AND A RAT MODEL ARE USEFUL BIOMARKERS OF THE DISEASE

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Keywords: nitrotyrosine, biomarkers, lymphocytes

Background: Peroxynitrite formation has been largely considered as one of the possible mechanisms involved in amyotrophic lateral sclerosis (ALS) pathogenesis. Once formed peroxynitrite can exert its toxic effect converting tyrosine to 3-nitrotyrosine (NT) leading to protein nitration and oxidation. Increased levels of NT and NT-modified proteins have been found in the central nervous system (CNS) of patients and mouse models of familial ALS (fALS), suggesting a possible use of nitrated proteins as biomarkers. In ALS there is increasing evidence that alterations that occur in motor neurons are also present in peripheral cells such as lymphocytes.

Objectives: In search for protein biomarkers of ALS from accessible samples, we decided to analyze whether tyrosine nitrated proteins were increased in peripheral blood mononuclear cells (PBMC) of sporadic ALS (sALS) patients, at two degrees of disease severity, and of the mutant SOD1 transgenic rat model at different disease stages.

Methods: We analyzed nitrated proteins in PBMC samples by a proteomic approach, using two-dimensional gel electrophoresis in combination with MALDI-TOF/TOF mass spectrometry. The level of protein nitration was evaluated by immunoblot with the anti-NT antibody or with a specific anti-nitrated actin antibody.

Results: We identified for the first time the major over-nitrated proteins in PBMC from patients and rats at different disease stages. In the rats, the increased level of nitrated proteins was detected already at a presymptomatic stage. Among them, actin, ATP synthase and vinculin overlap between sALS patients and the rat model. Interestingly, in a previous study, we have found that actin and ATP synthase were over-nitrated in the spinal cord of a mouse model of fALS before disease onset, suggesting the possible involvement of these proteins in motor neuron degeneration. We are now developing and testing specific antibodies for over-nitrated proteins and in particular for actin that we found nitrated in patients and animal models.

Discussion and Conclusions: This work indicates that nitrative stress is not restricted to spinal cord, the tissue specifically affected by the disease, but extends to the periphery and is found in SOD1-linked animal models and sporadic cases. On the basis of these observations it is possible to speculate that protein nitration could be the effect of converging pathogenetic mechanisms and therefore worthwhile to be studied further from a mechanistic point of view. Moreover, nitrated proteins can be considered as promising candidate biomarkers for early diagnosis of ALS and an anti-NT-actin antibody may be an interesting tool for clinical evaluations.

C20 ALSBANK™ VIRTUAL BIOREPOSITORY PLATFORM WITH DYNAMIC SAMPLE SEARCHES FROM HETEROGENEOUS DATA SOURCES

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Keywords: BioRepository, heterogeneous data sources, biomarkers

Background: Controlled and secure access to biosamples and ability to conduct dynamic searches based on clinical information from multiple databases is crucial to translational research. Most BioRepository platforms to not support association of existing inventory with clinical and phenotypic information collected during clinical trials and biomarker studies while meeting regulatory requirements.

Objective: To build a distributed Web-based platform for virtual BioRepository to manage sample collection and support dynamic search criteria capabilities across heterogeneous data sources from clinical trials and biomarker studies to ALS.

Methods: The ALSBank™ platform has been built on top of the existing PharmaENGINE™ system utilizing the "state machine" model for workflow configuration and customization; it provides the following capabilities to researchers:

- Manages BioRepository operations and inventory across collection sites
- Provides role-based access to samples inventory information
- Manages accessibility to multiple clinical and trial databases
- Queries information from multiple clinical sources utilizing their data dictionaries
- Enables online sample requests across multiple BioRepositories

Results: The ALSBank™ Virtual BioRepository platform that enables secure and controlled Web access for investigators to search, request and receive biosamples and de-identified clinical data from virtual multiple BioRepositories is currently deployed at the Neurology Clinical Trials Unit of MGH and is available to all member sites of the NEALS consortium.

- Repositories of biological samples from three research institutions, MGH, Emory University and University of Pittsburgh, are virtually connected
- Individual vial bar-coding schema with unique clinical trials identifier is implemented
- Individual vial's location is known down to the cell number in a storage box
- Bar-coded storage boxes are tracked to freezers at their respective institutions
- The ALSBank™ system allows dynamic searches across multiple clinical databases with heterogeneous data dictionaries
- Ad hoc variables could be created and linked to the databases
- Searches could be saved and re-used for new samples as needed

Conclusion: The ALS researchers from different institutions may join the ALSBank™ community to create a virtual BioRepository with secure controlled access to biosamples and clinical information collected during multiple clinical trials and biomarker studies.

SESSION 3B CLINICAL PHENOTYPES AND DISEASE PROGRESSION

C21 MRI-BASED NEUROIMAGING AS A SURROGATE MARKER IN ALS AND OTHER MOTOR NEURON DISORDERS: PROSPECTS AND PITFALLS

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Keywords: magnetic resonance imaging, surrogate marker, computational neuroanatomy

Computerised magnetic resonance imaging (MRI)-based techniques are increasingly used to analyse the structural and functional pathoanatomy of the brain in neurodegenerative diseases such as ALS and other MND *in vivo*. Besides volumetric approaches in regions-of-interest and magnetic resonance spectroscopy, especially whole brain-based techniques are accepted advanced neuroimaging tools for cross-sectional or longitudinal investigations, such as morphometric 3-D T1-weighted MRI analysis (e.g. voxel-based morphometry) and diffusion tensor imaging (DTI) which provides insights into white matter microstructure at group or individual level. Most promising for the *in vivo* mapping of structural MRI-based computational neuropathology are multiparametric protocols with multiple neuroimaging techniques in combination. For a correlational or direct analysis of the functional impact, it has been shown that the inclusion of covariance analysis with clinical parameters or the co-registration with task-specific functional mapping (functional MRI) might give complementary information.

The application to ALS but also to other MND entities with different clinical phenotypes of upper or lower MN affectation has successfully been used to identify lesion patterns of central motor and extra-motor areas and thus provided the option to map the alterations of MND patients' brains *in vivo* - at least at group level. That way, it has been possible to define pathomorphological grey matter or white matter 'fingerprints' of various types of MND. Furthermore the post-processing techniques allow for a quantitative comparison of MRI data from MND patients with controls, including tract-based statistics of DTI data. It has to be considered, however, that with respect to being used as a diagnostic tool at the individual patient´s level, the above-named techniques face severe limitations in applicability, sensitivity and specificity. One important aim in MND beyond gaining a deeper understanding of the disturbed neuroanatomical and functional networks will be the establishment of (combined) MRI protocols as a ('dry') biomarker (i) in natural history studies with MND patients who are well-defined in all aspects of their clinical presentation and (ii) in potential disease-modifying multi-center trials. For that purpose, there is not only a need for a higher number of longitudinal studies but also for more uniformity in the acquisition and especially post-processing of computer-based neuroimaging data in order for the methods to be both reproducible and valid. This aim is to be realised in large quality-controlled multi-national databases.

C22 NEW DIAGNOSTIC CRITERIA FOR PRIMARY LATERAL SCLEROSIS: A PROSPECTIVE VALIDATION STUDY

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Keywords: PLS, HSP, prognosis

Background: Diagnosis of primary lateral sclerosis (PLS) is by exclusion and current diagnostic criteria include a disease duration of ≥4 years to exclude ALS and a negative family history to exclude hereditary spastic paraparesis (HSP). However, sporadic presentation of HSP is not rare while genetic testing for many HSP forms is currently not available. We have previously shown that differentiation of sporadic HSP from PLS based on clinical characteristics is unreliable, although bulbar region UMN symptoms may support diagnosis of PLS.

Objectives: To prospectively assess the diagnostic and prognostic value of new criteria for clinical diagnosis of PLS, incorporating different levels of certainty of diagnosis of PLS instead of sporadic HSP.

Methods: Ninety one patients were included and classified according to our new PLS diagnostic criteria. Inclusion criteria were a gradually progressive, adult-onset (≥18 years), UMN syndrome, of ≥6 months duration. Exclusion criteria were a positive family history, clinical or electrophysiological evidence of generalized LMN involvement, and evidence of other causes.

Results: Included were 22 patients with 'suspected PLS' (duration <4 years), 30 with 'possible PLS' (duration ≥4 years, UMN signs only in legs), 13 with 'probable PLS' (duration ≥4 years, UMN signs in arms and legs), and 26 with 'definite PLS' (duration ≥4 years, UMN signs in at least bulbar region), according to our criteria. After a median follow-up of 3.0 years (range 0.1–4.3), 27 (30%) had shifted to a more certain PLS diagnosis, 7 (8%) fulfilled criteria for ALS, and 3 patients (3%; 2 'possible PLS', 1 'probable PLS') were re-diagnosed with another disorder (2 HSP, 1 corticobasal degeneration (CBD)). Conversion to ALS did not occur in 'possible PLS'. In 3 'suspected PLS' patients conversion to ALS was associated with clinical LMN signs and with typical short survival (median 2.7 years, range 2.3–3.7). Overall, rate of disease progression was variable and not related to EMG findings. At last verification 21 patients had died: 4 died of

ALS, 1 of CBD, 11 died indirectly related to PLS, and 5 died of unrelated causes.

Discussion: Our new PLS diagnostic criteria help to identify patients in whom PLS diagnosis is less certain, either because they may still evolve to typically progressive ALS (in 'suspected PLS'), or because they may still have sporadic HSP instead of PLS (in 'possible PLS' and 'probable PLS'). Criteria for 'definite PLS' identify patients very unlikely to have sporadic HSP, although some of these patients may develop mild LMN signs or even fulfill EMG criteria for ALS, which in our study was not clearly associated with more rapid clinical deterioration.

Conclusions: These new PLS criteria have diagnostic and prognostic value in the differentiation between sporadic HSP, typical ALS, and PLS.

C23 A RETROSPECTIVE ANALYSIS COMPARING FAST AND SLOW ALS PROGRESSION AND STUDYING FUNCTIONAL DECLINE AFTER INITIATING PEG AND BIPAP

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Keywords: ALSFRS-R, PEG, BiPAP

Background: ALS Functional Rating Scale-Revised (ALSFRS-R) is a commonly used outcome measure that may predict survival in ALS clinical trials. Determining which of the 12 ALSFRS-R questions best predicts survival is valuable information for both trials and clinical practice. Bi-level Positive Airway Pressure (BiPAP) and Percutaneous Endoscopic Gastrostomy (PEG) improve ALS survival but their impact on patients' functional status is unclear.

Objectives: To determine patient characteristics and early ALSFRS-R answers that best predict future disease progression, and to compare the rate of functional (ALSFRS-R) and respiratory (FVC) decline before and after BiPAP and PEG placement.

Methods: This is a retrospective study of (519) ALS patients who participated in one of the following trials: celebrex ($n = 300$), creatine ($n = 104$), arimoclomol ($n = 84$), and CoQ10 ($n = 31$). A linear mixed effects model was used to distinguish fast progressing (FP) and slow progressing (SP) patients compared to mean ALSFRS-R decline over time. A t-test was used to compare continuous variables and a Chi-Squared test was used to compare categorical baseline variables. A Cochran-Armitage test was used to test for trends of baseline ALSFRS-R questions. A stepwise-Cox regression model was used to investigate the relationship between ALSFRS-R answers and survival. A linear mixed effects model was used to compare ALSFRS-R and FVC slopes before and after starting BiPAP and PEG. Only subjects with at least two ALSFRS-R and FVC measurements before and after BiPAP or PEG were included in this analysis.

Results: There were 41% fast progressors (FP) and 59% slow progressors (SP). Site of onset and time from symptom onset to diagnosis were different between the two groups ($p = 0.03$) and ($p = 0.004$), respectively. There was no significant difference in other baseline characteristics including age, gender, family history, baseline weight, or riluzole intake. Answers to ALSFRS-R questions at baseline visit were all significantly different between SP and FP except for handwriting ($p = 0.41$) and respiratory insufficiency ($p = 0.17$). Trial dropout rate of FP (41%) was 3.2 times more than SP (13%). Out of the 12 ALSFRS-R questions, climbing ($p = 0008$), writing ($p = 0.04$), and salivation ($p = 0.04$) were the only predictors of survival. ALSFRS-R rate of decline was 0.35 units/month faster after BiPAP onset ($p < 0.0001$) and 0.32 units/month faster after PEG placement ($p < 0.0004$). These results remained significant after omitting ALSFRS-R "Swallowing" and "Respiratory insufficiency" questions for PEG ($p = 0.01$) and BiPAP ($p = 0.001$) analysis. The rate of FVC decline was not significantly different after BiPAP ($p = 0.09$) or PEG ($p = 0.94$).

Discussion and Conclusion: Slow ALS progressors are more likely to enroll in and complete clinical trials. Most ALSFRS-R questions are good early predictors of disease progression. Climbing, writing, and salivation ALSFRS-R questions are good predictors of survival and none of the "respiratory" questions are. Functional decline measured by ALSFRS-R is faster after PEG or BiPAP use.

C24 TARDBP GENE MUTATION IN ALS PATIENTS: A GENOTYPE-PHENOTYPE CORRELATION STUDY

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Keywords: familial ALS, TARDBP, phenotype

Objectives: To describe the phenotype of ALS patients with TARDBP gene mutations and present genotype-phenotype correlations.

Background: In approximately 10 to 20% of ALS cases, at least 2 cases are present within the pedigree, defining it as familial ALS (FALS). In 25% of those FALS cases a SOD1 mutation has been described as responsible for the disease. Recently, we and others have described TARDBP gene mutations in both sporadic and familial cases. While SOD1 mutations may modulate phenotype with rapid or slow evolution and with lower limb onset in the majority of the cases, the phenotype of ALS patients with TARDBP mutations has not been described, to date.

Methods: We describe phenotype and genotype of 9 ALS patients presenting with TARDBP gene mutations and their phenotype-genotype correlations.

Results: The five men and four women studied had the following characteristics: mean age of onset 58 ± 10 (range 46–78), 7 out of 9 had a upper limb onset, the disease was sporadic in 5 and within the 4 FALS cases (a mother and her son carried the same TARDBP mutation), mean disease duration was 71 months ± 53 (range 10–158), two patients are alive after 8 years and one of them had a tracheostomy after 5 years of ALS. Clinically all patients had both upper and lower motor neuron signs in 2 or 3 regions, with typical EMG features, without sensory conduction abnormalities. Three patients had transcranial magnetic stimulation (TMS) and single fibre EMG (SFEMG). In all cases these examinations were abnormal. Out of the 9 patients, 6 different mutations were found. Comparison between mutations, FALS and sporadic cases suggested that FALS cases have an earlier onset and a much longer course of ALS (103 months vs 39).

Discussion: Compared with classical ALS, patients carrying TARDBP mutation have few different characteristics. The apparently most significant difference lies in the site of onset as an upper limb onset is present in 7 out the 9 patients. However, mutated patients cannot be clinically distinguished from classical ALS cases. ALS is more slowly evolving in familial cases with TARDBP mutations, as already suggested in other FALS cases with or without SOD1 mutations. To date, our series is too limited to conclude that some specific TARDBP mutations are associated with a more rapid or slow ALS in opposition with that shown with SOD1 mutations.

C25 TACKLING THE CHALLENGES OF ALS FROM A FAMILIAL PERSPECTIVE

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Keywords: SOD1, pre-symptomatic, biomarkers

Background: Amyotrophic lateral sclerosis (ALS) is a disease that remains shrouded in mystery. With the exception of rare mutations in genes such as superoxide dismutase (SOD1), the etiology of ALS is unknown. Little is known about environmental risk factors for ALS. Other than electromyography and motor unit number estimation, there are no biomarkers for ALS that might permit early diagnosis or be useful in monitoring disease progression or therapeutic response. Apart from riluzole there are no effective therapies. We contend that the systematic study of asymptomatic SOD1 positive (SOD1+) individuals, the only population known to be at risk for developing ALS and SOD1+ ALS patients, offers several unique opportunities to unravel many of the mysteries of this disease.

Objectives: To (1) characterize the pre-symptomatic phase of ALS, (2) identify environmental factors that may modify the age of disease onset and which may be relevant to the risk of sporadic ALS, (3) develop biomarkers for early diagnosis, and (4) evaluate the safety and efficacy of arimoclomol in SOD1+ familial ALS patients.

Methods: We address these objectives through two parallel studies. The first, the Pre-familial ALS (Pre-fALS) study, is a prospective observational study. The second is a phase II/III randomized controlled trial (RCT) of arimoclomol, which targets a relevant pathophysiological mechanism and is effective in the SOD1 mouse when administered even after symptom onset. For Pre-fALS we offer genetic testing and counseling in order to identify and recruit presymptomatic SOD1+ individuals from familial ALS pedigrees. Study participants are evaluated annually using clinical, neurophysiological and neuroimaging techniques and environmental exposure assessment; biospecimens are also collected. For the RCT, we recruit individuals with recently diagnosed SOD1+ ALS. These individuals are randomized to receive arimoclomol or placebo and are followed serially over a 12-month period. The trial employs a novel 'remote evaluation' approach whereby most study procedures are performed in the study participant's home.

Results: As of May 2009, 68 subjects have been enrolled in Pre-fALS. Most participants who were previously unaware of their SOD1 gene mutation status, have elected to undergo genetic counselling and to learn their results. Over half of these subjects have completed their initial set of evaluations and some have already returned for their first annual follow-up visit. Twelve subjects have been screened for the arimoclomol trial and so far two subjects have been randomized.

Discussion and Conclusion: Pre-fALS is unique insofar as it prospectively follows asymptomatic people at risk for ALS using a broad range of investigative modalities. The arimoclomol RCT employs a novel 'remote evaluation' approach and focuses exclusively on the SOD1+ population. Progress to date is evidence of the feasibility of these studies. Although currently in their early stages, they hold promise to provide unique insights into disease biology and therapy.

SESSION 4A FUNCTIONAL GENOMICS

C26 WHOLE-GENOME EXPRESSION PROFILING IN MOTOR CORTEX AND SPINAL CORD OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: gene expression, microarray

Amyotrophic lateral sclerosis (ALS) is a fatal disorder caused by the progressive degeneration of upper and lower motor neurons. Despite identification of disease-linked mutations, the etiology and pathogenesis of ALS are still elusive. By whole-genome expression profiling using Agilent 44k whole genome expression arrays and stringent significance tests we have characterized genes and pathways de-regulated in the motor cortex (43 samples) and spinal cord (31 samples) of patients with sporadic ALS. We used a two-sided one-way ANOVA using Welch's t-test and Benjamini and Hochberg False Discovery Rate procedure as a multiple testing correction. For pathway analysis we used the Gene Map Annotator and Pathway Profiler (GenMAPP) 2.0 software package. Mutations in SOD1, Ang, Tardbp, Fig4 and Chmp2b were excluded. Gene and sample numbers investigated allowed the association of genomic changes not only to disease status and tissues examined, but also to other phenotypic characteristics, such as gender and length of survival. Our findings highlight the importance of immune responses (e.g. the complement cascade and HLA), cytoskeletal (tubulin and neurofilament subunits) and signal transduction dysfunction (cell proliferation and vesicle processing), offering suggestions for new potential ALS therapies.

C27 CHROMOGRANIN VARIANTS AS RISK FACTOR AND MODIFIER OF DISEASE ONSET FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: risk factor, modifier gene, chromogranin

Background: Recently, chromogranins were reported to interact specifically with mutant forms of superoxide dismutase (SOD1) that are linked to amyotrophic lateral sclerosis (ALS). Chromogranins can also interact with oxidized WT SOD1 but not with intact WT SOD1. This interaction is further supported by the recent report of a colocalization of chromogranins with SOD1-immunopositive aggregates in motor neurons of sporadic ALS cases.

Objective: Our objective was to screen the chromogranin B gene (CHGB) for the possible existence of sequence variations associated with ALS.

Methods: We have analyzed the frequencies of sequence variants of the CHGB gene in a total of 610 ALS patients and 561 controls from three different countries, France, Canada (French Canadian origin) and Sweden. PCR primer pairs were designed from genomic DNA to amplify each exon of the CHGB genes including the flanking splice sites. Products were PCR-amplified, checked on agarose gels, and then sequenced using the forward primer for all of the amplicons.

Results: Two novel missense variants not present in controls have been identified in the CHGB gene. Moreover, two other variants were overrepresented in ALS cases compared with controls. The combined CHGB variants were present in 11.2% of total ALS patients as compared to 4.1% in controls (odds ratio 2.9; 95% confidence interval 1.7–4.9) and conferred a 2.7 folds greater relative risk to develop the disease. Of particular interest was the finding of a common CHGB variant that was significantly increased in ALS patient chromosomes when compared to the control population ($P < 0.0001$). This missense variation is predicted to have a functional effect and to alter the protein structure. The frequency of this particular variant in ALS patients of French origin (France and Quebec) was around 17% as compared to 4% in controls, conferring a relative risk of 4.1 times greater to develop disease. Furthermore, this CHGB variant lowers the age at onset by almost a decade in both SALS and FALS cases.

Discussion: These results suggest that some CHGB variants are important risk factors for ALS as well as modifiers of disease onset. The risk conferred by CHGB variants is quite robust being comparable to the 3-fold over-representation of APOE-ε4 isoform in Alzheimer's disease patients. Our study reveals for the first time the existence of sequence variants in CHGB that increase the risk for ALS and that can associate with earlier onset of disease.

C28 CELLULAR IMPACT OF HFE H63D ALLELIC VARIANT ON ALS

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Keywords: HFE, cellular stress, SOD1

Background: In 2004, we were the first to report that a specific polymorphism in the hemochromatosis gene HFE, H63D, was over-represented in ALS. The prevalence (30%) of the H63D allele that we and the other groups reported was the second most frequent genetic variation reported in ALS. However, how H63D HFE might increase the vulnerability to ALS is as yet unknown.

Objectives: Here we continue our efforts in this new line of research to understand the mechanisms for how the presence of the H63D polymorphic allele of the HFE gene impacts ALS.

Methods: We developed a cell model by introducing tetracycline inducible expression system in a human neuroblastoma SH-SY5Y cell line to determine the cellular effects of the HFE H63D allele.

Results: The expression of H63D HFE mutant protein initially triggered the unfolded protein response (UPR), as revealed by the elevated level of the major UPR sensor, BiP/GRP78. This response was followed by down-regulation or cleavage of some UPR sensors, such as IRE1α and ATF4, and the activation of Caspase 3, indicating apoptosis was being promoted as a result of persistent ER stress. In addition, H63D HFE protein expression was associated with a higher level of protein oxidation, compared to wild-type protein. In the tetracycline inducible HFE expressing SH-SY5Y cell line, the presence of H63D HFE also resulted in the decreased expression and activity of Cu/Zn superoxide dismutase 1 (SOD1). Consistent with the cell culture model, we found that in ALS patients, individuals carrying H63D HFE allele have 40% less SOD1 expression in the muscle tissue compared to those with WT HFE.

Discussion and Conclusions: Together, our data suggest that the H63D HFE gene variant activates UPR, promotes apoptosis and increases oxidative stress. The presence of H63D HFE was associated with the decreased expression and activity of Cu/Zn SOD1. These results demonstrated that H63D HFE had multiple cellular consequences which might induce neuronal vulnerability. This novel cell model harboring H63D HFE may be instrumental for clarifying the relationship of this allelic variant to the disease mechanisms in ALS.

C29 HFE POLYMORPHISMS AFFECT GLUTAMATE REGULATION

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Background: Glutamate neurotoxicity is one of the leading theories for neurodegeneration in ALS and the H63D HFE gene variant appears with increased frequency in patients with ALS. We developed cell and animal models that express different HFE polymorphisms to explore the mechanisms behind the relationship between HFE H63D and ALS.

Objective: To determine if the presence of the HFE H63D genetic allele, a putative disease modifier in ALS, can alter glutamate homeostasis.

Methods: Human neuroblastoma cells (SHSY5Y) were stably transfected with either wildtype (wt), H63D or C282Y variants of the HFE gene. Human astrocytoma cells were selected based on expression of HFE polymorphisms. A knock out/knock in mouse model was generated to express the H67D gene variant (mouse equivalent of the H63D variant). A series of analyses were performed to determine the effect of HFE polymorphisms on the relative amounts of glutamate secretion and uptake, and intracellular calcium levels that could impact glutamate release.

Results: Cells expressing H63D HFE have 57% greater calcium-induced glutamate secretion over a 24 hour period. Expression of wt HFE in the SH-SY5Y cells was associated with glutamate uptake 2.5-fold higher than cells expressing H63D HFE. Minocycline was examined because of its antioxidant properties and to test our hypothesis that HFE genotype will influence treatment outcomes. Minocycline treatment significantly increased glutamate uptake in vector cells and significantly decreased glutamate uptake in wt HFE cells but had no effect on glutamate uptake in the H63D cells. HFE-dependent effects on glutamate uptake were confirmed in astrocytoma cell lines. Because the cellular analyses indicated abnormalities in the glutmatergic system in the H63D cells, we determined the expression of the cystine-glutamate antiporter (xCT) which is associated with glutamate release and oxidative stress in the mouse model. At 6 months of age, the brains from wt, heterozygous and homozygous H67D mice were harvested. There is an increase in brain iron levels in both heterozygotic and homozygotic mice compared to wt. xCT is two-fold higher in the H67D mouse brains compared to age and sex matched controls. These data are consistent with increased glutamate release from cells and are suggestive of increased oxidative stress.

Discussion: This study demonstrates HFE H63D may promote glutamate toxicity in both cell and animal models. The ability of minocycline to affect glutamate uptake differed by HFE genotype and implicate oxidative stress in the HFE effects. These results have significant implications with respect to the underlying pathogenic relationship between HFE gene variants and ALS. Furthermore, the data suggest that response to Riluzole or other therapeutic agents which alter glutamate homeostasis could be influenced by HFE genotype and argue for stratification of clinical outcomes data according to HFE genotype.

C30 THE ROLES OF RNA EDITING AND CALCIUM-PERMEABLE AMPA RECEPTORS IN MOTOR NEURON DISEASE

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Keywords: RNA editing, GluR2

Background: One plausible hypothesis for selective neuronal death in sporadic ALS is AMPA receptor- mediated excitotoxicity. The Ca^{2+}-permeability of AMPA receptors is largely determined by RNA editing at the GluR2 Q/R site, which is catalyzed by the ADAR2 enzyme. Professor Shin Kwak and colleagues have reported that there is deficient RNA editing at the GluR2 Q/R site in individual spinal motor neurons in Japanese sporadic ALS patients, suggesting that this is tightly linked to the etiology of sporadic ALS.

Objectives: To confirm the results in Japanese patients we obtained spinal cords (T6) of seven ALS and seven normal control subjects from the Sheffield Brain Tissue Bank in UK.

Methods: We dissected single spinal motor neurons using the PALM Laser Microdissection System and then extracted total RNA, generated RT-PCR products covering the GluR2 Q/R editing site and an adjacent intron, digested with restriction enzyme BbvI which cuts at the unedited but not the edited Q/R site and calculated the editing efficiency by measuring the relative efficiency of restriction digestion.

Results: The editing efficiency at the GluR2 Q/R site varied between 0% and 100% in 82 single spinal motor neurons of ALS patients and also in 96 from normal control subjects. There is no significant difference between the ALS and control sample sets.

Discussion: We confirm that incomplete RNA editing at the GluR2 Q/R site occurs in single spinal motor neurons of ALS patients as reported. However incomplete RNA editing was

also found in normal control subjects stored in the Sheffield Brain Tissue Bank in UK. Our results for control subjects differ dramatically from the Japanese group who reported the only direct data that complete editing of the GluR2 Q/R site occurs in normal human motor neurons. We expect that reduced Q/R site editing retards splicing at the proximal splice junction of the GluR2 pre-mRNA - this may have led to a biased failure pattern in RT-PCR amplifications from single motor neurons in earlier work. The control samples from Sheffield were from patients who died after hospitalization and there may have been some hypoxia in these patients. Therefore we obtained one spinal cord (C1 or C2) from the Edinburgh Sudden Death Bank in UK. However these motor neurons also showed incomplete RNA editing at GluR2 Q/R site.

Conclusions: We have verified the Japanese report that RNA editing at the GluR2 Q/R site is not complete in ALS motor neurons but our data so far suggests that less-than-complete RNA editing is normal in human motor neurons.

C31 MOTONEURON-SPECIFIC CHANGES IN THE EXPRESSION OF THE DRUG EFFLUX TRANSPORTER P-GLYCOPROTEIN THROUGHOUT DISEASE PROGRESSION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS: IMPLICATIONS FOR ALS THERAPY

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Keywords: drug resistance, efflux transporter, p-glycoprotein

Background: Despite numerous animal drug trials to interfere with different pathogenic pathways, pharmacological approaches to cure the mouse that models amyotrophic lateral sclerosis (ALS) have so far failed. This suggests either yet undefined crucial pathogenic mechanisms or a frank pharmacoresistance to treatments in these mice. Multi-drug transporters mediate drug responses by influencing pharmacokinetics and pharmacodynamics and, among different drug transporter systems, the multi-drug resistance efflux transporter P-glycoprotein (P-gp; mdr1; ABCB1) extrudes a broad range of xenobiotics from cells and confers chemoresistance resulting in a poor clinical outcome. Physiologically, P-gp is predominantly localized to capillary endothelial cells in the CNS and, to a lesser extent, in parenchymal and perivascular astrocytes. Although kainate-triggered seizures lead to P-gp expression in neurons and upregulation in the cell types mentioned above, little is known about P-gp localization and distribution under pathological conditions of the spinal cord.

Objectives: Our objective is to examine P-gp distribution and expression in the spinal cord of SOD1-G93A mice during ALS disease progression.

Methods: At multiple disease stages, spinal cords of SOD1-G93A and age-matched control mice were removed, cryosectioned, and immunofluourescently labeled with an antibody that recognizes the C terminus of P-gp (H241; Santa Cruz) and the motoneuron specific antibody SMI-32. Transcript levels of P-gp from whole spinal cord samples of a separate SOD1-G93A cohort were also quantified at these disease stages using qRT-PCR.

Results: Here we report the first evidence that P-gp is expressed in motoneurons. In controls, $\sim$60% of lumbar motoneurons express P-gp. Further, P-gp expression increases in lumbar motoneurons throughout disease progression, with $\sim$70% and $\sim$76% of motoneurons expressing P-gp in symptomatic and endstage animals, respectively. P-gp mRNA levels increase throughout the course of the disease, starting at onset, by $\sim$20% and progressing to a $\sim$50% increase at a later stage of disease.

Discussion and Conclusions: Since P-gp extrudes xenobiotics from cells, our results imply that spinal cord motoneurons may actively shunt pharmacological agents into the extracellular fluid of the spinal cord to facilitate drug extrusion by active transport systems in the blood cerebrospinal fluid barrier. Thus, P-gp localization to motoneurons may act as a secondary barrier to drug penetration into the nervous system. In pathological conditions, upregulation of efflux transporters in CNS parenchyma may provide a cellular compensatory response to extrude increased levels of endogenous toxins resulting from the disease. These combined mechanisms could constitute a powerful barrier that impedes drug delivery to the diseased spinal cord in ALS.

SESSION 4B CLINICAL ELECTROPHYSIOLOGY

C32 UNDERSTANDING THE PATHOPHYSIOLOGY OF ALS

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Keywords: hyperexcitability, excitotoxicity, transcranial magnetic stimulation

Establishing the presence of a combination of upper and lower motor neurone abnormalities affecting the same region is critical for the diagnosis of ALS. As such, a clear focus of current ALS research relates to the identification of the pathophysiological processes that may induce such an environment.

In terms of the lower motor neurone involvement, recent studies have identified widespread dysfunction of axonal membrane, with increased persistent Na^+ conductances, and abnormalities of fast paranodal and internodal slow K^+ channel function. Together, these changes contribute to the peripheral hyperexcitability typical of ALS, leading to the almost inevitable symptoms of cramps and fasciculations.

When considering upper motor neuronal involvement, ALS is characterised by progressive degeneration of the corticospinal tract, running from the brain through the spinal cord, to anterior horn cells that control all voluntary movements. Of relevance, most ALS aetiologies have been linked to an excitoxicity cascade, with excessive activation of glutamate receptors triggering destruction of motor neurones.

Recent studies that assessed cortical excitability using novel threshold tracking transcranial magnetic stimulation (TMS), demonstrated that cortical hyperexcitability was an early feature in sporadic ALS. Cortical hyperexcitability was also evident in familial patients with mutations in superoxide dismutase (SOD-1), suggesting similar pathophysiological processes operate in both sporadic and familial ALS patients.

Some have argued that reduction of short interval intracortical inhibition in ALS, and thereby the development of cortical hyperexcitability, was not a primary event, and merely reflected downregulation of inhibitory control compensating for spinal motor neurone loss. To consider this possibility, threshold tracking TMS studies undertaken in patients with spinobulbar muscular atrophy (SBMA) revealed normal cortical excitability when compared to ALS patients. Further comparison of SBMA to a pure lower motor neurone flail-arm ALS variant, established the presence of cortical hyperexcitability in the latter cohort of patients. Together, these findings indicate that the development of cortical hyperexcitability does not simply represent downregulation of intracortical inhibitory processes in ALS.

Longitudinal studies in asymptomatic SOD-1 mutation carriers recently established that cortical hyperexcitability may develop prior to the clinical onset of ALS. Reduction of short interval intracortical inhibition in ALS appears to be determined by a combination of a loss of inhibitory cortical interneurone and glutamate-mediated downregulation. These findings in SOD-1 mutation carriers were further supported in the G93A SOD-1 mouse model, where degeneration of spinal cord motor neurones occurred secondary to dysfunction within central nervous system motor pathways. Taken in total, these more recent findings may lend further support for the 'dying forward' hypothesis, with corticomotoneurones inducing anterograde excitotoxic motoneuron degeneration. From a therapeutic perspective, neuroprotective strategies aimed at preserving the integrity of intracortical inhibitory circuits, as well as antagonizing excitatory cortical circuits, may provide novel therapeutic targets in ALS.

C33 THE NEW ELECTRODIAGNOSTIC CRITERIA FOR ALS: A RETROSPECTIVE STUDY

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Keywords: Awaji, diagnostic, criteria

Background: Recently, a modification of the electrodiagnostic criteria for ALS was proposed by the Awaji commission in order to improve the sensitivity without diminishing the specificity. The commission did so, mainly by introducing 2 new concepts: 1) to accept neurophysiological evidence of lower motor neuron involvement as equivalent to clinical evidence; 2) to accept fasciculation potentials as an equivalent to fibrillation potentials or positive sharp waves. As a consequence 'probable-laboratory-supported ALS' was no longer accepted as a separate category.

Objective: To evaluate the effect of the new diagnostic neurophysiological Awaji criteria for the diagnosis of ALS.

Methods: In a retrospective study we reviewed both clinical and neurophysiological data of 213 consecutive patients, that visited our outpatient clinic for motor neuron diseases, from October 2006 until December 2008.

Results: Using the original criteria 93 patients were diagnosed with ALS: 13 definite, 38 probable, 14 probable-laboratory-supported, 28 possible. An alternative diagnosis was present in 120 patients. Using the new criteria 93 patients were diagnosed with ALS: 13 definite, 53 probable, 27 possible. Seven patients changed from possible to probable. Of the patients diagnosed as probable-lab-supported, 8 changed to probable, and 6 changed to possible. No patients with an ALS-mimic changed to the diagnosis ALS.

Discussion: The new criteria for ALS do not result in a loss of specificity and can potentially improve the sensitivity by 16% percent (15 patients). However, the fact that not all patients with 'probable-lab-supported ALS' (requiring UMN signs in one region) fullfilled the new criteria for probable ALS (requiring two regions), nearly eliminated the diagnostic yield. For this, we propose an adaptation to the new criteria in the sense that patients fulfilling the presence of LMN in two regions and UMN in one region are also categorized as probable ALS.

Conclusions: The Awaji modifications of the El Escorial Criteria do not result in a loss of specificity and might substantially improve the sensitivity.

C34 MODIFIED INCREMENTAL MOTOR UNIT ESTIMATION IN A LONGITUDINAL NATURAL HISTORY STUDY OF SUBJECTS WITH ALS

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Keywords: motor unit number estimation, physiology, biomarker

Background: Motor unit number estimation (MUNE) has been used in previous ALS trials to assess the progress of motor axon loss and consequent re-ennervation of denervated muscle fibers by surviving neurons. A number of different MUNE methods have been described, but each method has limitations that have limited utility. Recently, we developed a method of MUNE that incorporates elements of both the incremental and multiple point stimulation technique, is rapidly performed, and has excellent test-retest variability. This method (called modified incremental MUNE) has been employed in a multicenter natural history study of electrical impedance myography and MUNE in ALS subjects at 8 participating sites.

Objectives: To determine test retest variability in normal subjects across multiple data collection sites, and assess the changes in MUNE and associated measures in subjects with ALS.

Methods: After informed consent was obtained, subjects underwent MUNE evaluation of a distal hand muscle that was felt to be moderately affected by axon loss. Either the ulnar or median nerve was stimulated at three sites that were rigidly defined, and three incremental responses were obtained at each site. The response of the third increment was added together for all three sites and divided by nine to obtain an estimate of single motor unit potential (SMUP) amplitude. This value was divided into the maximum compound motor unit action potential to yield the MUNE value. Subjects were studied approximately every 2–3 months for up to one year.

Results: Thirty-three normal subjects were tested twice at 8 centers, after rigorous training of the evaluators. Average MUNE for normal subjects was 229 (SD: 93) for 33 median nerves, and 211 (SD: 68) for 14 ulnar nerves. Average test retest variability was 13% for median nerve studies, and 17% for ulnar nerve. Fifty one subjects with ALS have been studied longitudinally at the 8 participating sites. At onset average MUNE was 50 (range 3–192), and declined consistently over the course of the study. Average SMUP amplitude was 210 uV at study onset, and increased as disease progressed.

Discussion and Conclusions: Modified incremental MUNE can be reliably performed in a multicenter trial and yields results that show a consistent decline of MUNE over time, with corresponding increase in SMUP amplitude. This technique can be easily performed on any EMG machine, and requires no specialized software. These attributes make this method attractive for use in multicenter therapeutic trials.

C35 A PROSPECTIVE AND BLIND STUDY OF THE TRIPLE STIMULATION TECHNIQUE IN THE DIAGNOSIS OF ALS

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Keywords: magnetic stimulation, upper motor neuron, prospective diagnostic study

Background: Demonstration of upper motor neuron (UMN) signs in several regions of the body increases the accuracy of the diagnosis of ALS, but validated tests are lacking. The importance of demonstrating subclinical UMN involvement in another region may even increase with the Awaji criteria. The triple stimulation technique (TST) combines transcranial magnetic stimulation of the motor cortex with nerve stimulation at intervals appropriate for collision. In open studies TST has a high sensitivity.

Objective: To study the utility of TST in the diagnosis of ALS in a prospective study.

Methods: Fifty-nine patients were recruited to undergo TST in addition to the standard work-up for suspected motor neuron disease. The neurologist making the diagnosis was blinded to the TST results. Primary outcome was the number of abnormal TST results in patients with possible ALS. The positivity rate was also converted to the number needed to test with TST (NN-TST) for one extra diagnosis of ALS.

Results: Fifty patients underwent TST. In 19 of these patients, the amplitude ratio was abnormal. In the total patient group (n = 59), 18 patients had a motor neuron disorder but did not fulfill criteria for 'probable' or 'definite' ALS, and in 4 of these patients the TST was abnormal (NN-TST: 4.5). Most TST abnormalities were found in patients with clinically evident central motor disorders, resulting in an overall NN-TST of 15. In one patient the TST was erroneously interpreted as abnormal. TST findings were normal in all patients with inclusion body myositis and peripheral nerve disorders.

Conclusion: This prospective and blind study confirms open studies of TST in the evaluation of ALS. We suggest that abnormal TST can be used to arrive at a diagnosis of 'probable' or 'definite' ALS in patients lacking UMN signs in the upper extremities. We suggest that the equivalence of neurological signs and neurophysiological abnormalities, one of the major principles of the Awaji consensus, is valid for the upper and lower motor neuron.

C36 THE UTILITY OF SINGLE FIBER ELECTROMYOGRAPHY IN THE DIFFERENTIAL DIAGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS AND CERVICAL SPONDYLOSIS

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Keywords: single fiber electromyography, cervical spondylosis, diagnosis

Background: In the El Escorial revised criteria for diagnosis of amyotrophic lateral sclerosis (ALS), single fiber electromyo-

graphy (SFEMG) is regarded as a technique which can demonstrate the chronic partial denervation.

Objective: To assess the utility of single fiber electromyography (SFEMG) in the differential diagnosis of amyotrophic lateral sclerosis (ALS) and cervical spondylotic radiculopathy and myelopathy.

Methods: SFEMG was performed in extensor digitorum communis muscles (EDC) of three groups of patients, including 61 ALS patients with findings of cervical spondylosis on MRI, 59 ALS patients with normal MRI of cervical spine, and 55 patients with cervical spondylotic radiculopathy and myelopathy. The parameters of SFEMG between different groups were compared.

Results: The mean jitter were $(81.2 \pm 25.9)\mu s$, $(91.6 \pm 32.4)\mu s$, $(40.9 \pm 11.8)\mu s$ in ALS patients with findings of cervical spondylosis on MRI, ALS patients with normal MRI of cervical spine, and patients with cervical spondylosis respectively. P_{50} of the percentage of jitter $>55\mu s$ were 73%, 80% and 5% in the three groups respectively; P_{50} of the percentage of block were 10%, 20% and 0% in the three groups respectively. Fiber densities were 2.9 ± 0.5, 2.9 ± 0.6 and 2.4 ± 0.6 in the three groups respectively. There was no significant difference in those parameters of SFEMG between the ALS with findings of cervical spondylosis and those with normal MRI. There was significant difference in those parameters of SFEMG between the patients with ALS and those with cervical spondylosis ($P < 0.01$). In 18 patients with ALS, conventional EMG studies showed active and chronic denervation in only one region at the first visit, including 10 in lower limbs, 5 in one upper limb, 3 in bulbar region. SFEMG was performed in EDC which had normal MRC and EMG. SFEMG showed increased FD in 16 patients, increased jitter in 13 patients and impulse block in 6 patients.

Discussion and Conclusions: SFEMG showed significantly increased jitter and block in ALS whether there are MRI findings of cervical spondylosis or not, which can help to differentiate ALS from cervical spondysotic radiculopathy and myelopathy.

SESSION 6A MOTOR NEURON BIOLOGY

C37 SONIC HEDGEHOG SIGNALING AND THE CONTROL OF MOTOR NEURON AND VENTRAL INTERNEURON GENERATION

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Keywords: Sonic Hedgehog, embryonic spinal cord, MN development

Neuronal subtype specification in the vertebrate neural tube is one of the best-studied examples of embryonic pattern formation. Distinct neuronal subtypes are generated in a precise spatial order from progenitor cells according to their location along the anterior-posterior and dorsal-ventral axes. Underpinning this organization is a complex network of extrinsic and intrinsic factors. Particularly well understood is the mechanism that determines the generation of motor neurons and interneurons that control motor output in ventral regions of the spinal cord. In these regions of the nervous system, the secreted protein Sonic Hedgehog (Shh) acts in graded fashion to organize the pattern of neurogenesis. This is a dynamic process in which increasing concentrations and durations of exposure to Shh generate neurons with successively more ventral identities. Interactions between the receiving cells and the graded signal underpin the mechanism of Shh action. Of note, the transcriptional regulation of genes induced or repressed by Shh signaling plays an essential role in shaping the graded readout. Thus the accurate patterning of the neural tube and the specification of motor neurons and the other neuronal subtypes characteristic of this region relies on the continuous processing and constant refinement of the cellular response to graded Shh signaling.

C38 FROM MOTOR CORTEX TO MUSCLE: SPECIES DIFFERENCE IN MOTOR PATHWAYS

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Understanding the comparative functions of the corticospinal tract is of direct relevance to our understanding of how animal models can advance knowledge of the human motor system and its disorders, including ALS/MND. I will present different lines of evidence to demonstrate significant species differences in both the organisation and function of the corticospinal tract. There are also some important differences in corticospinal projections from different cortical regions within a particular species and these projections are known to support different functions. Therefore any differences in the organisation of corticospinal projections across species may well reflect differences in their functional role. These differences have probably evolved to support key elements of sensorimotor behaviour. It is now widely recognised that the corticospinal tract, in addition to its classical role as a descending motor pathway, also subserves a number of other functions. One of these is in descending control of somatosensory afferent input: this appears to be one the earliest corticospinal functions to have evolved. Another essential feature is the modulation of spinal reflex activity and interaction with central pattern generators. The capacity for direct influence over motoneurons is a relatively late evolutionary feature: direct cortico-motoneuronal (CM) projections are unique to primates, are well developed in the most dexterous non-human primates and most highly elaborated in humans. The excitatory input from these CM projections varies widely across muscle groups, being strongest to the perioral muscles and the muscles acting on the digits of the upper and lower extremities (and especially the thumb). The highly developed nature of the CM system in humans and its pattern of projections to different muscle groups have implicated this system in some forms of ALS/MND. Studies in non-human primates will continue to be needed for understanding some special features of the human motor system, including the control of skilled hand movements and higher capacities such as tool use. These movements are often particularly vulnerable to neurological disease such as ALS/MND.

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SESSION 6B CLINICAL TRIALS AND TRIAL DESIGN

C39 CEFTRIAXONE IN ALS: RESULTS OF STAGES 1 AND 2 OF AN ADAPTIVE DESIGN SAFETY, PHARMACOKINETIC AND EFFICACY TRIAL

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Keywords: clinical trials, ceftriaxone, pharmacokinetics

Background: The study aim is to determine the efficacy and safety of treatment with ceftriaxone in ALS. Ceftriaxone increases expression of the astrocytic glutamate transporter, EAAT2 and protects from glutamate, and superoxide dismutase mediated toxicity.

Objectives: We propose a novel study design strategy of nonstop drug development. We will apply data from an intermediate analyses of cerebrospinal fluid (CSF) penetration and safety toward the develoment of an efficacy study. The CSF pharmacokinetics of ceftriaxone in subjects with ALS (STAGE 1) will be followed by a safety and tolerability study for 20 weeks (STAGE 2), and then a full efficacy trial (STAGE 3). The first two STAGES are complete.

Methods: In STAGE 1, 66 subjects at ten clinical sites were enrolled, equally divided into groups receiving intravenous placebo, ceftriaxone 2g or ceftriaxone 4g daily. A plasma and CSF pharmacokinetic study was conducted at day 7. All subjects continued treatment after the STAGE 1 study and entered STAGE 2. The DSMB and part of the Steering Committee reviewed the data and decided that study should proceed to STAGE 3, at 4g/day, studying a total of 600 randomized research participants at 62 centers in US and Canada. New subjects will be randomized in a 2:1 fashion to receive treatment of (1) ceftriaxone or (2) placebo. Participants will remain in study until 52 weeks after the last participant is randomized. The co-primary outcome measures are survival and rate of change in ALSFRS-R. Secondary outcome measures include change in vital capacity, evaluation of upper and lower extremity muscles using hand-held dynamometry and quality of life and the long-term safety of ceftriaxone.

Results: STAGES 1 and 2 have been successfully completed. Ceftriaxone was found to have a volume of distribution of 13.8 liters and a plasma half-life of 8.6 hours. Plasma and CSF concentrations were closely correlated. CSF trough levels at both doses (2g and 4g) exceeded the prespecified target trough level of 1μM. At the 4g daily dosage, modeling predicted that CSF levels would stay above 1μM for 72 hours, enabling consideration of drug holidays for subjects if needed. At 20 weeks of treatment, subjects from both active treatment dose levels met the pre-specified criteria for tolerability.

Discussion: The complexity of this study has required a team of clinical investigators from several fields, toxicologists, pharmacokineticists, project managers, data managers, and biostatisticians. We successfully achieved the goals of the first two STAGES of the trial and now are proceeding with STAGE 3.

Conclusion: The novel activities of cephalosporins provide a unique opportunity to evaluate a single agent aimed at several pathways relevant to the pathophysiology in patients with ALS.

C40 KNS-760704-CL201, PART 1: A 12-WEEK PHASE 2 STUDY OF THE SAFETY, TOLERABILITY, AND CLINICAL EFFECTS OF KNS 760704 IN ALS SUBJECTS

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Keywords: KNS-760704, clinical, neuroprotection

Background: KNS-760704 is a novel drug being developed for the treatment of ALS. This was a 12-week, double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of (6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride (KNS-760704) in ALS patients. Subjects received 50, 150, or 300mg KNS-760704, or placebo daily for 12 weeks. A secondary objective was to evaluate the effects of KNS-760704 on clinical function measures including the ALS Functional Rating Scale-Revised (ALSFRS-R) and vital capacity (VC).

Methods: Key eligibility criteria were: possible to definite ALS diagnosis, $\leq$2 years since ALS symptom onset, and vital capacity $\geq$ 65% of predicted. Concomitant riluzole at a stable dose was permitted. Safety evaluations were conducted at Baseline and Weeks 1, 2, 4, 8, and 12. Clinical function was assessed at Baseline and Weeks 4, 8, and 12. Blood samples were collected in a subset of subjects after at least 1 week of dosing for a pharmacokinetic (PK) sub-study. Subjects completing the 12-week placebo-controlled study period (Part 1) were eligible to enter a double-blind, randomized active safety extension phase (Part 2). Reported here are Part 1 study results; Part 2 of the study will be completed by Nov 2009.

Results: A total of 102 subjects were enrolled at 20 US centers; 98 subjects (96%) completed the study. Twenty-three subjects participated in the PK sub-study. There were no deaths or treatment-related serious adverse events over 12 weeks. Two subjects (1 placebo; 1 300mg) discontinued due to adverse events (AEs). There were no per-treatment-group

differences in the overall incidences of AEs, treatment-related AEs, or pre-specified clinically significant vital sign, ECG, or laboratory abnormalities. Two subjects (300mg) had reversible CTC grade II neutropenia, one of whom restarted treatment with KNS-760704 in Part 2 without recurrence. Mean/median changes from baseline to endpoint in ALSFRS-R total scores were −3.6/−4.0 (placebo), −5.0/−3.0 (50mg), −3.3/−2.5 (150mg), and −2.2/−2.0 (300mg). Relative to the decline in the placebo group, the 300mg group showed a 39% improvement in mean ALSFRS-R change from baseline to endpoint and a 50% improvement in median ALSFRS-R change from baseline to endpoint. Mean changes from baseline to study endpoint in upright VC (% predicted) were −13.1 (placebo), −10.8 (50mg), −6.4 (150mg) and −10.7 (300mg). Pharmacokinetics were linear over the range of doses tested and $t_{1/2}$ was ∼8 hours.

Discussion: KNS-760704 was safe and well-tolerated in this study. Encouraging dose-related and time-dependent improvements in ALSFRS-R total scores relative to the placebo group observed in this study suggest that KNS-760704 may slow the rate of motor function loss in ALS subjects. Further evaluation of KNS-760704 in larger and longer studies is merited.

C41 STAR TRIAL: SUB-ANALYSIS OF AN INTERNATIONAL, MULTI-CENTER, PLACEBO-CONTROLLED STUDY OF AVP-923 (DEXTROMETHORPHAN/QUINIDINE) FOR THE TREATMENT OF PSEUDOBULBAR AFFECT (PBA) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Background: Pseudobulbar affect (PBA, also known as pathological laughing and crying) is characterized by exaggerated/involuntary emotional outbursts and occurs in patients with degenerative and traumatic neurological conditions including ALS. Incidence of PBA in ALS patients is particularly high (up to 50%). Dextromethorphan (DM), an NMDA-receptor antagonist and sigma-receptor agonist, reduces excitatory neurotransmission and may improve PBA. Quinidine (Q) inhibits CYP2D6 isoenzyme, slowing DM metabolism producing higher and sustained DM plasma levels. The STAR Trial has been designed to evaluate the safety and efficacy of AVP-923 for the treatment of PBA in ALS and MS patients. We report here the sub-analysis for ALS patients.

Methods: Eligible ALS patients were randomized in a 1:1:1 ratio into one of the three blinded treatment arms, to receive AVP-923-30/10 (30 mg DM/10mg Q), AVP-923-20/10 (20mg DM/10mg Q) or placebo b.i.d. for a 12-week period. Patients who completed the blinded phase of the study were eligible to participate in an open-label safety extension receiving the highest dose (AVP-923-30/10 b.i.d.) for an additional 12-week period. The main criterion for eligibility was a score of 13 or greater in the CNS-LS (Center for Neurologic Studies-Lability Scale). Primary efficacy endpoint was the number of PBA episodes recorded daily in a patient's diary; secondary efficacy endpoints were CNS-LS, NPI-Q (Neuro-Psychiatric Inventory), BDI-II (Beck Depression Inventory), SF-36 (Health Status Survey) and CSI (Caregiver Strain Index). Safety was assessed by physical examination, vital signs, clinical laboratory tests, ECGs, and oxygen saturation. Patients were asked to record any adverse experience and all medications taken in their diaries.

Results: From a total of 251 screened ALS patients, 197 (78.5%) were enrolled over a 15-month period at 68 clinical sites in USA, Argentina and Brazil. Frequent reasons for ineligibility were concomitant depression and disallowed medications. Interim demographic and baseline data are available for 148 randomized ALS patients. Gender distribution: 42% females, 58% males. Median age was 57.2 years old (Min: 25.3; Max: 75.12). Median time from ALS diagnosis at baseline was 11 months (1; 111). Median time from PBA diagnosis in ALS patients was 6 months (1; 111). CNS-LS median score at baseline was 20 (13; 35). The last patient was enrolled on 31 March 2009 with last follow-up scheduled at the end of June, 2009.

Conclusions: The STAR trial represents the largest and longest double-blind, randomized AVP-923 study to date for the symptomatic treatment by dextromethorphan/quinidine of pseudobulbar affect in ALS patients. Safety and efficacy data on primary and secondary endpoints will be available upon presentation.

C42 REGULATORY DOCUMENTS MANAGEMENT PLATFORM: EFFICIENCY, SCALABILITY, REGULATORY COMPLIANCE AND STANDARDIZATION IN CLINICAL TRIALS IN ALS

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Keywords: clinical trials management, project management, TREAT ALS

Background: The Neurology Clinical Trials Unit (NCTU) at MGH serves as Coordination Centre for The Northeast ALS consortium's Clinical Trials Network with the goal to "translate research advances into clinical trials for patients with ALS." Several multi-site clinical trials and biomarker studies in ALS are coordinated simultaneously.

Objective: To design and develop a comprehensive and customizable Web-based platform that brings efficiency, scalability, regulatory compliance and standardization in conducting clinical trials and biomarker studies.

Methods: Web-based TREAT ALS Platform was designed and built to manage clinical studies in ALS. It is comprised of several modules that allow the Coordination Centre team to:

- Collect and maintain real-time information on: site equipment and capabilities and site members qualifications
- Follow Regulatory Documents (RD) during the life cycle of a clinical trial: from trial initiation to FDA submission.

The Platform allows users to:

- Define trial-sepcific roles and corresponding required RD
- Identify site member participants for trial-specific roles
- Provide RD for approval and regulatory compliance
- Verify sites' RD completion and readiness

- Generate reports for missing and expired documents
- View site and trial participants readiness in Virtual Regulatory Binder
- Flag missing and expired documents
- Accelerate trials' initiation and subjects' enrollment
- Track IRB Submissions across multiple sites and protocol versions
- Reduce time spent on IRB submissions
- Track protocol deviations
- Increase accuracy of information
- Improve regulatory compliance

Results: The Regulatory Documents Management platform:

- Deployed at the Neurology Clinical Trials Unit at MGH
- Available to all member sites of the NEALS consortium
- Site and member information collected and maintained for 130 sites
- Sites notified via e-mail to update and upload missing & expired RD
- Collected site information utilized in site selection for future trials
- Improved regulatory compliance
- Ability to track site metrics
- Faster trial startup and enrollment
- Utilized for Phase III trial of Ceftriaxone in ALS (58 sites) and Phase II trial of Lithium in ALS (36 sites)

Conclusion: All new clinical research NEALS-based initiatives will utilize the TREAT ALS platform. This platform adds efficiency, standardization and regulatory compliance to the conduct of ALS trials.

Acknowledgements: We thank The ALS Association for its continuing support.

C43 THE EFFECT OF INCLUSION CRITERIA ON OUTCOMES: INTERPRETING RESULTS IN HISTORICAL PLACEBO CONTROLLED TRIALS

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Keywords: trial design, historical controls, patient demographics

Background: Using historical placebo controls could reduce sample sizes and costs, and speed enrolment in ALS trials. The WALS Lithium study is the largest to use historical controls and was designed to test the large effect reported in an Italian study on Lithium. The large response would be easily refuted if the outcome appears only similar to historical controls, or confirmed if the outcome appears much superior. Detecting smaller effects, however, using this trial design is still challenging. Results from past trials have differed with respect to the observed rates of decline in placebo groups, and even small differences affect assumptions about what "true" rate of decline should be used for historical comparisons. Understanding what factors affect outcomes, including variations in enrollment criteria, recruitment methods, disease timing, phenotypes, and demographics, and creating mathematical models to control for differences would be a key step in the interpretation of results.

Objectives: To explain the differences in prior placebo outcomes from different ALS clinical trials; and to present novel methods for analyzing trial data in historically controlled trials.

Methods: Using our historical placebo database (containing 748 patients) we studied rates of decline among different trials, and created mathematical models to control differences in baseline patient characteristics that could have affected outcomes.

Results: Data from 4 past trials that used the ALSFRS-R slope decline as an outcome were used. We found differences in the mean distributions of slopes during the first six months of follow-up (range: -0.80 to -1.03). We found the differences had the closest correlations with predicted rate of decline, initial forced vital capacity (FVC), and the duration of symptoms since onset. Studies that set cutoffs with lower FVC or longer symptom durations found greater rates of decline than those using more restrictive criteria. In addition, individual patients with longer disease durations, higher baseline FVC, and slower pre-trial estimated decline tended to progress slower. The effect of timing was important because distributions of disease durations varied from trial to trial, perhaps due to definitions of "timing" that affected enrolment or due to differences in healthcare demographics. When corrections to slopes were made using a linear model, the differences narrowed and were no longer statistically significant.

Discussion and Conclusions: Differing enrolment criteria will affect mean rates of decline in trials, thus influencing observations of "true" slope declines in ALS. Subtle effects on timing could relate to regional differences in healthcare or study inclusion criteria. By using linear models that correct for the differences between the enrolled cohorts, the interpretation of outcomes in historically controlled trials may be improved.

SESSION 7A EMERGING DISEASE MODELS

C44 IPS CELLS AS *IN VITRO* MODELS OF ALS

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Keywords: motor neuron differentiation, stem cells, drug testing

Research into ALS disease mechanisms, and the testing of candidate drugs, have been hindered by the inaccessibility of the cell types directly affected in human patients: motor neurons and surrounding glia. Since it is not feasible to sample these cells from human patients, we are exploring the potential of human stem cell-derived motor neurons. Our aim is to recreate in the culture dish the conditions that lead to ALS, using human cells with the same genotype as ALS patients. Several steps toward this goal have been accomplished. First, we have created multiple lines of induced pluripotent stem (iPS) cells from ALS patients and healthy controls. Second, we have shown that these iPS cells can be maintained as undifferentiated stem cells and then differentiated into spinal motor neurons. We are currently exploring means by which these iPS cells can be used to recreate the ALS disease process. Our results using human ES (embryonic stem) cells show that we can: (a) produce motor neurons of different subtypes by patterning with extrinsic factors; (b) generate enriched cultures of astrocytes; (c) detect toxicity of potential environmental factors for motor neurons; (d) validate survival assays for human motor neurons. Future research should determine whether iPS-MNs derived from ALS patients show different properties from control iPS-MNs in these or other respects.

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C45 EVALUATION OF FUNCTIONAL MOTOR NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPS) AND HUMAN EMBRYONIC STEM CELLS (HESC)

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Keywords: stem cells, disease model, neurodegeneration

Background: Stem cells have the potential to treat neurodegenerative disorders. Their value may also lie in the opportunity to model human diseases *in vitro*, for which animal and other cell culture models have not yielded an understanding of underlying disease mechanisms, or new therapeutic approaches. The excitement surrounding pluripotent stem cell (IPS) technology is predicated upon the potential to treat disease or injury with derivatives of patient-specific stem cells. While the reprogramming of human somatic cells to an embryonic state is fast becoming commonplace, the potential for the differentiation of reprogrammed cells to prospectively defined post-mitotic neuronal phenotypes remains challenging.

Objectives: Here, we evaluated whether the methods shown to be effective at generating motor neurons from human embryonic stem cells (HESCs) were similarly efficient in differentiating motor neurons from iPS.

Methods: Fibroblasts from human skin samples were cultured and directed toward stem cell lineage by expressing the four transcription factors *KLF4*, *OCT4*, *SOX2*, and *C-MYC*. Strains of IPS and hESC were differentiated in parallel using retinoic acid, sonic hedgehog and multiple growth factors. Maturation and lineage was followed using immunohistochemistry with neuronal and motor neuron specific markers, PCR of developmental and neuronal markers and patch clamping to assess single cell electrophysiology.
Motor neurons derived from ALS-subject IPS and hESC that express SOD1 mutations linked to familial ALS were analyzed for cell survival by manual cell counts and the distribution of intracellular SOD1 by immunocytochemical staining.

Results: The IPS and hESC appeared to follow a normal developmental progression when directed to motor neuron lineage expressing developmental stage-specific markers and neuronal markers such as TUJ1, ChAT, Islet1 and Hb9 at similar time lines. Importantly, the resulting motor neurons from both strains possessed cell-type specific electrophysiological properties.
When motor neurons from ALS subjects or from hESCs that express mutant A4V, G93A or I113T SOD1 mutants are evaluated for cell survival, all strains exhibit increased cell death when compared to controls, which was statistically significant on Student's t-test. In addition, when staining for intracellular SOD1 protein, IPS and hESC-derived motor neurons are positive for aggregated SOD1 inclusions.

Discussion: We demonstrate the feasibility of using IPS and transgenic hESC-derived motor neurons for *in vitro* modelling of motor neuron disease. Several typical features of motor neuron degeneration are present in our model system. Further evaluation will characterize the process of neurodegeneration in stem cell derived motor neurons in more detail to help optimize these cell model systems, which can aide in the study of disease pathways of ALS in sporadic and familial ALS and help screen for new therapies.

C46 A SOD1 ZEBRAFISH MODEL OF ALS SHOWS INDUCTION OF EARLY CELLULAR STRESS

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Keywords: animal models, zebrafish, HT screening

Background: Mutations in the SOD1 gene have been shown to cause classic ALS in numerous families. Transgenic mice over expressing mutant SOD1 suggest a dominant gain of function that is independent of SOD1 enzymatic activity but the mechanism of this toxicity is

currently under debate. One excellent approach to uncover mechanism is to identify interacting genes. However the ability to perform suppressor screens is limiting in mouse models due to the low number of animals that can be tested and their high costs. Thus, additional animal models of ALS would benefit the field.

Objectives: Our goal is to generate another vertebrate model of SOD1 FALS that is amenable to genetic analysis.

Results: We have developed a transgenic SOD1 overexpression model of zebrafish that carries disease causing sod1 mutation in the zebrafish SOD1 gene (SOD1)**.** The zebrafish and human SOD1 genes are very similar and 87% of the amino acids associated with causing ALS are conserved in the fish gene. A zebrafish BAC containing the genomic region of sod1 was used to clone a 20 kb fragment containing the SOD1 gene and flanking sequences for generating the transgenics. Founder (F1) fish were identified from F0 mating and the lines expanded. We show that like mice, the ageing adult transgenic fish show evidence of motor neuron loss, muscle atrophy and death. This demonstrates that zebrafish, like mice and humans, can mimic the ALS phenotype accurately, thus validating the use of zebrafish in studying ALS/MND.
In generating our transgenic lines, we incorporated the promoter for zebrafish heat shock protein 70 (hsp70) regulating the expression of the fluorescent molecule DsRed as a way to track our transgenic fish. We found that in SOD1 mutants, but not in SOD1 wildtypes, the hsp70 promoter was induced without heat exposure as indicated by DsRed expression. At this stage the mutant zebrafish embryos appear grossly normal and show no visible behavioural abnormalities as compared to wildtype SOD1 transgenics. The mutant sod1 specific heatshock response occurs as early as 24 hpf and occurs in the brain and spinal cord, the cell types affected in ALS.

Discussion: The sod1 zebrafish model of ALS mimics most aspects of motor neuron disease. The early embryonic readout of mutant SOD1 induced heatshock response could be very valuable for identification of chemical and genetic modifiers of mutant SOD1 activity and help in further understanding of ALS pathogenesis.

C47 FUNCTIONAL CHARACTERIZATION OF MUTANT TDP-43 IN ZEBRAFISH

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Keywords: TDP-43, transgenic zebrafish, pharmaceutical screening

Amyotrophic lateral sclerosis (ALS) is an adult-onset rapidly progressing neurological disorder characterized by loss of motor neurons, cells which control muscle movement. Approximately 10% of ALS cases are familial (FALS). Recently, we made the breakthrough discovery of a considerable number of TARDBP mutations in FALS and sporadic ALS (SALS) patients, the gene encoding TDP-43, the protein that aggregates in inclusion bodies from the spinal cord of ALS patients. For this project, we have developed a series of human TDP-43 cDNA constructs tagged at both the C-terminus and N-terminus which will be used to determine mutant and WT TDP-43 expression in cell lines, primary motor neurons and zebrafish embryos. Here we demonstrate that mutant TDP-43 causes a specific motor neuron disorder through a toxic gain of function since expression of mutant TDP-43, but not WT, causes selective motor neuron toxicity in primary spinal cord cultures. Further, expression of mutant causes a specific motor phenotype and motor neuron axonal defects in zebrafish embryos. On the other hand, decreased expression using anti-sense morpholino oligonucleotide (AMO) of TDP-43 yielded a similar phenotype in zebrafish; thus a specific loss of function may also be involved in the selective vulnerability of motor neurons to mutant TDP-43. A further characterization of these models will allow us to unravel molecular partners of mutant TDP-43 as well as to allow a better understanding of specific mechanisms of disease involved in mutant TDP-43 caused motor neuron degeneration. We are also generating TARDBP knock-outs in zebrafish as well as mutant TDP-43 transgenic fish lines. These lines will be functionally characterized to determine whether they properly model motor neuron disorders. These lines can then be tested for the screening of a large number of pharmaceutical compounds in transgenic zebrafish expressing mutant TDP-43, thus directly opening avenues for the development of therapies that could delay or prevent disease onset and progression in all ALS patients.

C48 TDP43 TOXICITY IN THE CHICK EMBRYO MODEL SYSTEM

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Keywords: chick embryo, TDP43, apoptosis

Background: The TAR DNA binding protein (TDP-43) is the major protein in ubiquinated inclusions (UBIs) in FTLD-U and ALS. A phosphorylated 25-kD C-terminal fragment of TDP-43 and high-molecular weight ubiquitinated aggregates are enriched in detergent resistant fractions of FTLD-U and ALS brains. We aim to use the chick embryo model system to understand the function of TDP43 protein *in vivo.*

Methods: We electroporated TDP-43^{WT}, TDP-43^{Q331K} and TDP-43^{M337V} DNA into the spinal cords of HH stage 14 chick embryos using in ovo electroporation. All the 3 TDP 43 constructs were cloned into vectors carring different tags (Myc+HA or GFP). SOD1^{G93A}-dsRed, SOD1^{WT}-dsRed electroporation was used as a positive control and GFP electroporation as the negative control. After 24 hours the embryos showing normal development were processed for frozen sectioning and then apoptotic cell death was observed using TUNEL assay. Fluroescent microscopy was used to establish the cellular localisation of the wild type and the mutant TDP-43 protein.

Results: SOD1^{G93A}-dsRed transfected neurons showed cell death as demonstrated by TUNEL staining (number of embryos analysed = 36/36). We also observed a decrease in the population of motor neurons (stained for Islet1/2) on the transfected side of the embryo as compared to the non-transfected control side. TUNEL staining demonstrated that while GFP transfection results in some apoptotic cells, there is a significant increase in apoptotic nuclei in spinal cord sections of embryos electroporated with N-terminal HA and C-terminal Myc tagged TDP-43^{WT}, TDP-43^{Q331K} and TDP-43^{M337V} constructs. A two-tailed t-test of GFP vs HA-TDP-43^{WT}-Myc gave a p-value of 0.1252; GFP vs HA-TDP-43^{Q331K}-Myc gave a p-value of 0.0179 and GFP vs HA-TDP-43^{M337V}-Myc gave a p-value of 0.0079. In order to confirm that the Myc and HA tags were not causing the increased apoptosis, we carried out electroporation of GFP tagged TDP-43^{WT}, TDP-43^{Q331K} and TDP-43^{M337V}. Once again, TUNEL staining showed an increase in the number of apoptotic nuclei in embryos electroporated with either

TDP-43^{WT}, TDP-43^{Q331K} or TDP-43^{M337V} as compared to those electroporated with pEGFPC1. A two-tailed t test of GFP vs GFP-TDP-43^{WT} gave a p-value of 0.0337; GFP vs GFP-TDP-43^{Q331K} gave a p-value of 0.0026 and GFP vs GFP-TDP-43^{M337V} gave a p-value of 0.0045. The mutant forms of TDP 43 showed pre-dominantly cytoplasmic localisation with nuclear inclusions while TDP 43^{WT} was predominantly nuclear. A two-tailed t-test of of HA-TDP-43^{WT}-Myc vs HA-TDP-43^{Q331K}-Myc gave a p-value of 0.0650; HA-TDP-43^{WT}-Myc vs HA-TDP-43^{M337V}-Myc gave a p-value of 0.0263; GFP-TDP-43^{WT} vs GFP-TDP-43^{Q331K} gave a p-value of 0.0120 and GFP-TDP-43^{WT} vs GFP-TDP-43^{M337V} gave a p-value of 0.009

Conclusions: Over-expression of not only TDP-43^{Q331K} and TDP-43^{M337V} but also, TDP-43^{WT} causes neurotoxicity in the chick embryo spinal cord. The mutant forms of TPD-43 localise in the cytoplasm and show nuclear inclusions while TDP-43^{WT} localises in the nucleus.

SESSION 7B SPIRITUAL CARE AND MEANING IN LIFE

C49 MEANING IN LIFE AND PERSONAL VALUES IN ALS: EMPIRICAL DATA AND CLINICAL RELEVANCE

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Keywords: meaning in life, Schedule for Meaning in Life Evaluation (SMiLE), spirituality

Loss of meaning in life (MiL) is known to be one of the main determinants of requests for hastened death. Empirical data with respect to personal values and MiL in ALS patients and their clinical relevance will be discussed.

In a first study, the relationship between personal values and individual quality of life was evaluated in 28 ALS patients and 36 patients suffering from advanced cancer (1). The most important values were benevolence, self-direction and universalism, whereas power, achievement and stimulation were the least important. Self-transcendence values were higher than self-enhancement values in all patients. Compared with healthy adults, palliative care patients scored significantly higher in benevolence and lower in self-enhancement values. There were no significant differences between ALS and cancer patients. The observed shift towards self-transcendence values may be related to coping processes of terminally ill patients.

In another study (2), MiL was assessed with a newly developed instrument for the evaluation of individual MiL. In the Schedule for Meaning in Life Evaluation (SMiLE), the respondents list 3 to 7 areas which provide meaning to their life before rating the current level of satisfaction and importance of each area. Indices of total satisfaction (IoS), total weighting (IoW), and total weighted satisfaction (IoWS, range 0–100) are calculated. Forty-nine ALS-patients were interviewed at the Interdisciplinary Center for Palliative Medicine, Munich University Hospital-Grosshadern. All open answers were assigned to 13 categories found in the representative survey (3). The results were compared to those of the representative sample ($n = 1{,}004$).

MiL was slightly lower in ALS-patients (SMiLE-Index = 76.6 ± 20.2) compared to the representative sample (83.3 ± 14.8; $p = 0.03$; $d = 0.4$). The areas of MiL mentioned most often were family (82%), partnership (61%), leisure activities (53%) and friends (39%). Partnership and leisure activities were listed significantly more often by ALS-patients compared to the representative sample, while work and health were mentioned significantly less often ($p < 0.001$ for each). This was shown by logistic regression analyses; age, marital status and education were included as additional predictor variables.

Overall MiL (SMiLE-Index) was slightly lower in ALS-patients compared to the representative sample. Health and work were less important for ALS-patients, relations to significant others, especially partnership and leisure time were listed more often. This could be due to "response shift" in the progress of the disease, i.e. a change of internal standards (4). Identifying ALS-patients who are at risk of losing their MiL and helping them to find alternative sources of MiL may be an important step towards preventing requests for hastened death.

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C50 EVIDENCE BASED SPIRITUAL CARE IN ALS: FACT OR FICTION?

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Keywords: measuring spirituality, spiritual care training, pastoral care

Spiritual care is an essential part of holistic medicine, yet the methods of intervention in professional spiritual care seem to be somewhat obscure. What exactly does a spiritual care provider do in existential interventions for people facing a life threatening illness? Is there any evidence spiritual care is based on?

The talk starts with a wide and open understanding of spirituality where religion may or may not be part of a patient's spirituality. The realm of spirituality includes issues of meaning, transcending, becoming, and relating, depending on the individual. The spiritual relates to essential issues in palliative care, e.g. quality of life, hope, coping, anxiety, and awareness of dying. Tools measuring spirituality work with varying constructs of spirituality, depending on the expected outcome. Especially with ALS patients, constructs include an understanding of spirituality as a resource to the patient, as an area of conflict or suffering, as a search for meaning while nearing the end of life. These constructs affect the way a multiprofessional team includes spiritual issues in care. Some studies show the importance of a patient's spirituality for treatment decisions at the end of life.

Spiritual Care, however, not only focuses on the spirituality of the patient, but also the needs and resources of relatives, and of care providers. Studies point out the importance of spirituality for care providers, raising the question whether training in spiritual care makes sense.

Since spiritual issues are an integral part of a whole person/whole care approach to medicine, the question remains to be answered, who (i.e. which profession) should provide spiritual care? Is spiritual care - performed by doctors - another tool that helps physicians to better connect with patients, to better understand them as people? Or should the provision of spiritual care be reserved to chaplains? In order to answer these questions, the talk will present findings from studies on pastoral care in palliative care settings in Germany, giving facts on actual spiritual care, and their inclusion in a multiprofessional approach to whole person care. Some cases with ALS patients will be discussed that put spiritual care into the frame of evidence based reality instead of fiction.

C51 SPIRITUALITY AND FAITH: MEANS FOR COPING WITH THE EFFECTS OF ALS/MND

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Keywords: spirituality, faith, coping

Background: Spirituality and personal faith are recognised as important factors contributing to quality of life amongst those diagnosed with ALS/MND, assisting individuals to develop perspective and influencing their decisions as they adapt to living with the disease (1).

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 role of spirituality and faith as a means of coping with ALS/MND.

Methods: We previously reported the processes used for locating published and unpublished personal illness narratives about life with ALS/MND (2). The 161 narratives identified were subject to content and thematic analysis. Data management was aided by Nvivo 7 software.

Results: There is frequent reference throughout the narratives to the power of spirituality to impact positively on the illness experience. Authors refer to the strength acquired from seeking a spiritual understanding of their circumstances. There is a sense of being used for a 'higher purpose', being seen as a spiritual example to others. Religious convictions are frequently strengthened following the diagnosis, bound up in a feeling of not being burdened beyond their ability to cope. Faith is regarded by some as a cornerstone of the ability to cope. Belief in an afterlife sustains many authors as they recognise the present time as a small part of a much longer time span.

Conclusions: It is evident within the narratives that people with ALS/MND tolerate distress through maintaining hope by belief in a divine entity and through connection with a higher being (3). Spirituality should be regarded as an important resource for coping with ALS/MND.

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C52 MANAGING DEATH IN ALS/MND: IDENTIFYING CAREGIVERS' PERSPECTIVES OF THE FINAL DAYS OF LIFE

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Keywords: caregivers, dying, advanced planning

Background: Family caregivers usually remain central to the provision of care throughout the illness trajectory. Yet, little is known about how they manage or cope with the dying process and the death of their relative with ALS/MND.

Objective: This study sought to understand the experiences and support needs of family caregivers, during their relatives' final days of life.

Methods: Longitudinal studies of family caregiving in ALS/MND were undertaken in Australia and United Kingdom. Primary caregivers participated in in-depth interviews at four month intervals throughout the illness trajectory, including the bereavement phase. Five cases from each country were purposively sampled for analysis. A process of open and axial coding was used to categorise the data and identify themes as they emerged from each caregiver's story.

Results: In the broadly similar socio-cultural structure of Australia and the United Kingdom, family caregivers in both countries expressed similar experiences of their relative dying. However, their construction of the dying process varied, exhibiting delicately nuanced differences in their experiences and their responses. In eight out of ten cases, patients were cared for at home until their last days of life. All the United Kingdom cases died in hospital/hospice. Three of the Australian cases died at home and two died in hospital/hospice. Conversations between the caregiver and the dying person, about end of life, were only evident in six out of ten cases.
The most prominent themes across all cases were dignity, emotional labour, advanced care planning, spiritual sustenance, reflective guilt, and redefinition. Caregivers were often unprepared for the rapid deterioration and sudden death of their relative, prolonging their sense of loss and disconnection.

Discussion and Conclusion: The increasing trend towards dying at home is both challenging and rewarding for family caregivers. The quality of life focus, prominent during the earlier phases of living with ALS/MND, can be easily over looked in the medicalised processes of dying. When caregivers reflected on the dying experience and said "we got it right", they lived with the positive affirmations of caregiving. However, if the dying experience was characterised by conflict about decision making, then negative memories are perpetuated.
This study demonstrated the need for an inclusive process of effective communication between the patient, the family and health and social care professionals, to prepare for dying. There is a need to develop communication skills to facilitate conversations that will support patient and family caregiver decision making and enhance their coping skills. While it is recognised that patients and caregivers have to be emotionally ready to have these conversations, the often unpredictable, degenerative nature of ALS/MND increases the need to facilitate preparation for dying in a timely manner.

SESSION 7C FUS MUTATIONS IN FAMILIAL ALS

C53 MUTATIONS IN THE RNA BINDING GENE FUS CAUSE FAMILIAL ALS

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Keywords: FALS, FUS, Mutations

Background: Familial ALS accounts for 5–10% of cases and recently mutations in the gene TDP-43 were described in both familial and sporadic ALS (1). Following this, genes with similar functions in the chromosome 16 linkage region (2) were prioritised for mutation screening.

Objectives: To identify the causative mutation in the chromosome 16 locus.

Methods: In addition to the family (F1) who had previously been linked to chromosome 16, we identified 197 suitable FALS index cases from the UK and Australia, who had previously been screened and found to be negative for mutations in known ALS genes (SOD1, ANG, VAPB, Dynactin, CHMP2B and TARDBP). Exons of genes of interest were amplified and subsequently sequenced using BDT v1.1 on an ABI 3100.

Results: A point mutation was identified in exon 15 of the gene Fused in Sarcoma (FUS or TLS) in all affected members of the F1 family. This 1561 C->T change in the gene was predicted to result in an arginine to cysteine change at position 521. In a survey of 197 FALS index cases we identified two further missense mutations in eight families. Post-mortem analysis of three cases with FUS mutations showed FUS-immunoreactive cytoplasmic inclusions and predominantly lower motor neuron degeneration. Cellular expression studies revealed aberrant localization of mutant FUS protein.

Conclusions: We have identified mutations in a new gene for familial ALS. FUS shares functional domains with TDP-43 and the identification of mutations in a second RNA binding protein indicates the importance of RNA metabolism to motor neurons.

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C54 FUS MUTATIONS IN A LARGE SERIES OF SPORADIC AND FAMILIAL ALS

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Keywords: FUS gene, missense mutations, synonymous mutations

Background: Recently, the fused in sarcoma/translated in liposarcoma (FUS/TLS) gene, located on chromosome 16p11.2, has been identified as a disease gene in familial amyotrophic lateral sclerosis (FALS).

Aim: The aim of this study was to further define the spectrum of FUS/TLS gene mutations in a large series of FALS and sporadic ALS (SALS) patients.

Methods: DNA samples from 52 index cases with FALS were collected from eight Italian ALS referral centres and from 1,749 SALS of American and Italian origin. The entire coding region was sequenced in all FALS and 276 SALS. As previous publications have implicated exon 15 as a hotspot for mutations within this gene, this exon was sequenced in an additional 1,473 SALS. Detected variants were evaluated in a control cohort consisting of 280 neurologically normal Italian subjects and 460 neurologically normal US subjects.

Results: Among the 1,749 SALS patients we found a total of 7 cases with missense mutations (0.4%). All mutations have been detected in exon 15: c.A1552G (leading to p.R518C) (1 case), c.C1561T (p.R521C) (1 case), c.C1561G (p.R521G) (2 cases), c.G1562A (p.R521H) (1 case), c.C1574T (p.P525L) (1 case), and c.C1575T (p.P525R) (1 case). Moreover, in 5 cases we found the synonymous mutation c.G1566A (p.R522R). None of these variants were present in controls.
Among 52 index cases of FALS we identified a heterozygous c.G1542C missense mutation in a family of northern Italian origin, and a heterozygous c.C1574T missense mutation in a family of Sicilian origin. Both variants are located in exon 15 encoding the RNA-recognition motif, and result in a substitution of an arginine with a serine in position 514

(p.R514S) and substitution of a proline with a leucine at position 525 (p.P525L) respectively. Overall, the two mutations accounted for 3.8% of 52 non-SOD1 and non-TDP43 index cases of FALS. A synonymous mutation in exon 15 (c.G1566A, p.R522R) of another FALS index case was also detected. None of these variants were present in controls.

Discussion: FUS/TLS gene mutations have been found in 4% of non-SOD1 non-FTD43 FALS patients and in less than 0.5% of apparently SALS patients. Two novel heterozygous missense mutations in exon 15 have been found (c.A1552G and c.G1574T); also a synonymous mutation (c.G1566A) not detected in healthy controls has been found in 5 SALS cases and 1 FALS case.

The other members of the ITALSGEN Consortium are: F Lombardo (Torino), I Ossola (Torino), A Calvo (Torino), C Moglia (Torino), S Cammarosano (Torino), K Maurinou (Milano), A Conte (Roma), M Luigetti (Roma), P Sola (Modena), I Bartolomei (Bologna), C Carlesi (Pisa), R Spataro (Palermo), P Paladino (Palermo), C Ricci (Siena), F Giannini (Siena), GL Mancardi (Genova), G Tedeschi (Napoli).

C55 IDENTIFICATION OF FUS/TLS GENE MUTATIONS IN A COHORT OF ITALIAN FALS PATIENTS

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Keywords: genetics, ALS6, FUS/TLS

Background: Approximately 10% of all ALS cases are familial (FALS). Mutations in *SOD1* and *TARDBP* genes account for ~20% and ~5% of all FALS cases, while variants in other genes (*ALS2*, *SETX*, *VAPB*, *DNCT1*, and *ANG*) have been described in isolated pedigrees. Recently, mutations in the *FUS/TLS* gene have been identified in ALS families with linkage to chromosome 16 (ALS6).

Objectives: To perform a genetic screening of *FUS/TLS* to assess the mutational frequency among Italian FALS families.

Methods: We sequenced all 15 coding exons of *FUS/TLS* in 94 unrelated Italian patients with a diagnosis of probable or definite ALS according to the El Escorial revised criteria and a positive family history for motor neuron disease. All the participating individuals were negative for *SOD1*, *TARDBP*, and *ANG* mutations.

Results: We found four missense heterozygous mutations in five FALS patients. Two mutations (p.R521G and p.R521C) have been previously identified and were observed in one and two cases, respectively. Two novel mutations, p.G156E and p.R234L, were found in one patient each, and were absent in a panel of 376 age- and ethnically matched healthy controls.

Discussion: Arginine 521 is located in the Nuclear Localization Signal (NLS) of FUS/TLS protein, and its substitution with a glycine or cysteine has been shown to result in an aberrant subcellular distribution and apparent aggregation of the mutant protein in the cytoplasm. The p.G156E and p.R234L mutations are located in the SYQG-rich and RGG-rich domains of the protein, whose biological functions are still unknown. We hypothesize, however, that p.G156E and p.R234L are pathogenic since the residues at these positions are highly conserved throughout evolution and because these variants were not observed in our control panel. The clinical phenotype of our mutated patients was characterized by a predominance of lower motor neuron signs, and by a symmetrical, proximal and axial weakness at onset. Interestingly, a single patient also developed frontotemporal dementia (FTLD).

Conclusions: Our study provides evidence that *FUS/TLS* mutations account for ~3% of all Italian FALS patients, representing the third most important identified cause of FALS after SOD1 and TARDBP. It also suggests that patients carrying *FUS/TLS* mutations may have an uncommon clinical phenotype, possibly including FTLD.

C56 NOVEL FUS MUTATIONS IN A LARGE COHORT OF FALS PATIENTS

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Keywords: FUS, mutation, genetics

Background: Amyotrophic lateral sclerosis (ALS) is a fatal progressive paralytic disease caused by degeneration of motor neurons. Most of the ALS cases are sporadic (SALS), and approximately 5–10% of ALS cases are familial (FALS). Mutations in the Cu, Zn superoxide dismutase (SOD1) are responsible for about 20% of the FALS cases and mutations in the TDP43 cause ALS in about 3% of FALS cases. Mutations in the other identified genes are rare. Recently, 14 mutations in the FUS have been found in 26 FALS cases among a combined cohort of 491 FALS cases. Most of these cases are of Caucasian origin. The FUS mutations in the other populations have not been studied. The clinical features of the patients with the FUS mutations are largely unknown.

Objectives: To explore the spectrum of FUS mutations in FALS.

Methods: Genetic variations of the FUS were determined by direct sequencing of all the 15 exons in 461 SOD1- and TDP43- negative FALS index cases, the largest single FALS cohort studied to date. Additionally, over 500 controls were analyzed for each genetic variant observed. Clinical data from the patients with the FUS mutations were compared to those from patients with known SOD1 mutations.

Results: We carried out comprehensive genetic and clinical analyses of the FUS mutations in a large cohort of FALS cases from different ethnic groups. We have analyzed all the

coding exons of the FUS in 461 FALS cases, the largest data set analyzed to date. All the FALS cases were excluded for SOD1 and TDP43 mutations. We identified 13 FUS mutations in 22 FALS index cases or families, among which seven mutations are novel. Most of the mutations are missense, but mutations involving small deletions, splicing and truncations were also found. These mutations are aggregated in two clusters in FUS, thus providing potential functional relevance of the ALS-linked FUS mutations. We also found that the patients with the FUS mutations appeared to have higher rate of the bulbar onset and shorter duration of the disease when compared with the patients with the SOD1 mutations.

Discussion and Conclusions: Our data have demonstrated that the FUS mutations are a common genetic cause in FALS patients of different genetic backgrounds. The prevalence of the FUS mutations in the non-SOD1 and non-TDP43 FALS is about 5% (4.77%), or approximately 4% in all of FALS cases. Thus, the FUS mutations appear to be the second most frequent genetic cause of FALS after the SOD1 mutations. Patients with the FUS mutations manifest higher rate of bulbar onset and shorter duration of the disease.

C57 FUS MUTATIONS IN BELGIAN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: FALS, FUS, dominant

Mutations in FUS were recently identified as a novel cause of familial amyotrophic lateral sclerosis (ALS). The frequency of occurrence of mutations in FUS in sets of familial ALS patients remains to be established. We therefore sequenced FUS in a Belgian set of familial SOD1-negative ALS patients. The 15 exons and exon-intron boundaries of FUS were sequenced in 26 familial ALS patients from 19 different families. We identified a R521H mutation that segregated with disease in a kindred of dominantly inherited ALS. Mutations in FUS are responsible for 3% of pedigrees or 5% of familial ALS patients in our cohort of familial ALS. These results show that mutations in FUS are a significant cause of familial ALS in Belgium.

C58 MUTATIONS OF FUS/TLS GENE IN A LARGE CHINESE PEDIGREE WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1 gene, liposarcoma (FUS/TLS) gene

Background: Amyotrophic lateral sclerosis (ALS) is the most common and fatal degenerative motor neuron disease in adults. Approximately 20% of familial ALS (FALS) have been found to have the Cu/Zn superoxide dismutase (SOD1) gene as the causative gene. Recently, 5% of FALS was reported to be caused by liposarcoma (FUS/TLS) gene mutation.

Objectives: To screen for mutations of FUS/TLS gene in a big ALS Chinese family without mutation of the SOD1 gene.

Methods: All living subjects who consented for this study were examined by at least two independent neurologists for the diagnosis of ALS (according to the El Escorial and revised El Escorial criteria). They also underwent electromyographic (EMG) examination to establish lower motor neuron (LMN) dysfunction in non-symptomatic body regions. FUS/TLS gene mutation screening was performed by directly sequencing after informed consent.

Results: This non consanguineous Chinese family consisted of 5 generations and had an autosomal dominant inheritance pattern. Twelve patients including 9 males and 3 females were found. Ten were dead. Mean onset age was 40 year-old. The duration of the disease is about 1.5 years. The cause of death is breathing difficulty. We found a mutation (R521C) of sarcoma/translated in liposarcoma (FUS/TLS) gene in the pedigree. However, two possible patients harbored two reported polymorphisms including rs741810 (p.G49G) in exon3 and rs1052352 (p.Y97Y) in exon 4 with absence of this mutation.

Conclusions: Our data supports the fact that SOD1 mutations are rare in Chinese familial ALS patients and indicates FUS/TLS mutations may be more common. However, variant intrafamilial phenotype in the family resulting from polymorphisms remains unknown.

C59 SCREENING FOR FUS MUTATIONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FUS, mutations, familial ALS

Background: Recently, mutations in the FUS gene were shown to be associated with familial amyotrophic lateral sclerosis. Here we investigate these mutations in cases of hereditary motor neuron disease (including ALS, PMA and SMA).

Objectives: To investigate FUS mutations in familial forms of motor neuron disease.

Methods: We included 51 families with a family history of motor neuron disease. All patients were screened and were negative for mutations in SOD1, ANG, VAPB and TARDBP, as well as deletions of SMN1 and CAG repeats in the androgen receptor gene. We used capillary sequencing of whole-genome amplified DNA using BigDye 3.1 chemistry on all 15 exons. Mutations will be confirmed in a separate PCR and sequencing reaction on genomic DNA. For exons containing mutations, at least 900 neurologically normal controls are sequenced to confirm ALS specificity.

Results: Several mutations which were previously described are also found in our experiments in 5 out of 51 index cases (9.8%).

Conclusions: We are able to replicate previous findings on FUS mutations in our patient population. Work is as of yet

ongoing and further research into clinical phenotype of these patients has yet to be carried out.

C60 MUTATION SCREENING IN TRANSLOCATED IN LIPOSARCOMA GENE IN PATIENTS WITH MOTOR NEURONE DISEASE

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Keywords: Translocated in Liposarcoma gene, FUS, RNA splicing and transport

Background: Familial motor neurone disease (MND) accounts for 5–10% of all MND cases. Recently mutation in the Translocated in Liposarcoma (TLS) gene has been identified as a cause of MND. TLS, also known as FUS, is a 53KDa widely expressed protein. TLS is a multifunctional protein, possessing distinct N-terminal transcriptional activating and C-terminal RNA binding domains and has been implicated in a number of cellular processes including transcriptional regulation, RNA splicing and transport, and maintainance of genomic stability. Previous neuropathological reports of TLS MND cases have demonstrated an absence of TDP-43 positive inclusions which is the major pathological change seen in sporadic and non-SOD1 familial MND.

Objectives: To identify the frequency, phenotype and molecular pathology of MND associated with TLS mutation.

Methods: DNA was extracted from CNS tissue and whole blood of 168 MND patients, including 49 familial cases. Intronic based primers were used to amplify the 15 exons of TLS/FUS. PCR products were bi-directionally sequenced and analysed using Sequencher software. Autopsy tissue from 4 cases with nucleotide substitutions was assessed for neuropathology.

Results: Two novel mutations (p.Arg524Trp and p.Gly507Asp), were identified in 2 familial and 1 sporadic MND patients. These changes were not identified in 295 control individuals. The phenotype in these individuals was of an upper limb onset with predominantly lower motor neurone involvement. Neuropathological study of two of these cases showed marked lower motor neurone loss and either absent or very few ubiquitinated inclusions and no TDP-43 positive inclusions.
Two further changes were identified (p.Gly228_Gly229insGly and pGly174ValfsX183) in 1 sporadic and 1 familial case. These changes were identified at low frequency in control cases (0.3%) raising the possibility that they may represent coincidental polymorphisms. Neuropathological study of these cases revealed lower motor neurone loss with TDP-43 positive inclusions. These neuropathological findings support the possibility that in these further cases, the MND phenotype was not due to the changes identified in TLS.

Discussion: Mutations in FUS/TLS have been found in a large cohort of MND cases, supporting this gene as a cause of MND. However, stringent screening of controls and demonstrable functional consequences of the "mutations" is necessary to ensure that the nucleotide changes identified are pathogenic for MND.

SESSION 8A PROTEIN REGULATION AND DEGRADATION

C61 MOLECULAR MECHANISMS FOR THE ATROPHY AND PROTEIN LOSS FROM SKELETAL MUSCLE UPON DENERVATION AND SYSTEMIC DISEASE

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Keywords: muscle atrophy, denervation, protein breakdown

Whether a muscle grows or atrophies depends on the balance between rates of protein synthesis and degradation. Much has been learned recently about the mechanisms for muscle wasting seen with nerve injury, disuse, and systemic diseases (e.g. cancer, cachexia, sepsis, fasting, excess glucocorticoids). In these diverse conditions, loss of muscle mass is due mainly to an acceleration of protein degradation, primarily by the ubiquitin proteasome pathway. In various types of atrophy, we have identified a common program of changes in the expression of about 100 atrophy-related genes which we have termed "atrogenes". Amongst the genes most dramatically induced are the ubiquitin ligases, atrogin-1 and MuRF1 whose induction is necessary for rapid atrophy. During atrophy, certain proteins that stabilize the myofibril are selectively ubiquitylated by MuRF1 and are lost differentially, which appears to facilitate disassembly of the thick filaments. MuRF1, however, is not critical in the degradation of thin filament components which require different ubiquitination enzymes.

In atrophying muscles, signaling by the IGF-1-p13K-Akt-FoxO pathway decreases, and activation of the FoxO family of transcription factors play a critical role in muscle atrophy. Activation of FoxO3 is necessary for muscle wasting upon denervation or fasting and by itself causes rapid atrophy. FoxO3 causes induction of many atrogenes including atrogin-1 and MuRF1, and dominant negative FoxO3 mutations can block denervation atrophy.

The possible importance of lysosomes in muscle atrophy has received little attention. Using selective inhibitors of the lysosomal and proteasomal pathways, we were able to demonstrate that FoxO3 coordinately activates both processes. Activated FoxO3 stimulates lysosomal proteolysis in muscle (and other cell types) by activating autophagy and induces the expression of many autophagy-related genes. These autophagy-related genes are induced similarly in mouse muscles atrophying due to denervation or fasting and causes loss of mitochondria. These findings are the first evidence for coordinated regulation of the proteasomal and lysosomal systems which serve complementary roles in degrading different muscle components during atrophy, the myofibrillar apparatus by the ubiquitin-proteasome pathway and mitochondria by autophagy.

Muscle contractions can retard atrophy, in part by enhancing the production in muscle of IGF-1, which stimulates protein synthesis and blocks FoxO activation. Exercise also enhances production of the transcriptional coactivator, PGC-1a, which increases mitochondrial content, and in muscle directly inhibits the FoxO-dependent activation of the atrophy program. When overproduced, PGC-1a and its homolog PGC-1b were found to reduce muscle protein degradation and denervation atrophy. However, pharmacological enhancement of PGC-1a by AICAR treatment of mice maintained mitochondrial content but did not reduce fiber atrophy. By contrast, other approaches to block atrophy (e.g. by blocking the myostatin-Smad pathway) seem very promising.

C62 VPS54 MUTATION IN WOBBLER MOUSE MND CAUSES TDP-43 MISLOCALIZATION AND PATHOLOGY AS IN ALS

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Background: The ubiquitously expressed nuclear TAR DNA-binding protein (TARDBP) 43 (TDP-43) has been implicated in the pathogenesis of ALS. Although *TARDBP* gene mutations occur in some patients with familial ALS, essentially all patients with sporadic and most with familial ALS contain TDP-43 which is insoluble, ubiquitinated, hyperphosphorylated, and mislocalized to the cytoplasm of motor neurons. Studying animal models with TDP-43 pathology not due to *TARDBP* mutations may provide insights into its pathogenic mechanisms in sporadic and non-*TARDBP* familial ALS. MND in the wobbler (wr) mouse results from a mutation in the vacuolar-vesicular sorting protein 54 (Vps54), which disrupts intracellular trafficking as occurs in human ALS. We have found TDP-43 pathology in the wr mouse.

Objectives: We will characterize the TDP-43 proteinopathy in wr MND and determine how closely it recapitulates this pathology in human ALS.

Methods: The presence of TDP-43 protein was examined by immunohistochemistry and immunoblotting in eight week-old wr cervical spinal cord (CSC), an age and location where pathology is prominent, in comparison to healthy littermates. One, two, and 20-week old mice were also examined immunohistochemically. Immunostaining of 30 μm-thick sections was performed for either TDP-43 alone or in relation to markers of pathology (e.g., ubiquitin) and cell type (e.g., ChAT). Relative amounts of TDP-43 immunoreactivity and its state of ubiquitination were examined in cytoplasmic and nuclear fractions of homogenized CSC. Soluble proteins in these samples were sequentially extracted to determine if TDP-43 was relatively insoluble, and ubiquitinated.

Results: TDP-43 immunoreactivity is mislocalized to the cytoplasm of primarily cholinergic CSC motor neurons as granular, globular, or filamentous inclusions where it becomes ubiquitinated, although it also appears increased in neuronal nuclei. Most prominent when motor neuron degeneration is advanced, intracytoplasmic TDP-43 reaction product increases just prior to onset of clinical disease (7–14 days old). Subcellular fractionation reveals a unique protein signature of increased TDP-43 immunopositivity in both the cytoplasmic and nuclear fractions of wr CSC, including ~25–30 kD bands

not present in the wildtype. Wobbler TDP-43 protein is also ubiquitinated and insoluble as in ALS.

Discussion: TDP-43 proteinopathy occurs in the wr mouse in a way similar to human ALS, including cytoplasmic mislocalization, ubiquitination, insolubility, and the presence of ~25–30 kD bands, which in ALS represent pathologic cleaved C-terminal fragments. These abnormalities in the wr involve not only the cytoplasm but also the nucleus, suggesting pancellular TDP-43 pathology which may have implications to ALS.

Conclusions: The wr model of MND provides a framework for investigating the pathogenic mechanisms of TDP-43 toxicity in the absence of *TARDBP* gene mutations, which represents the majority of ALS. Identifying how the wr Vps54 mutation causes TDP-43 proteinopathy may reveal similar pathogenic mechanisms in human ALS.

C63 ABNORMAL INTRACELLULAR STORAGE IN LOSS OF FUNCTION OF FIG4

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Keywords: Fig4, lysosomal storage, electron microscopy

Fig4, a phosphatase, regulates the level of PI(3,5)P2 and plays an important role in intracellular vesicular trafficking, particularly for the endosomes/lysosomes. Our previous studies have demonstrated that mutations in human Fig4 gene cause a recessive form of Charcot-Marie-Tooth disease type-4J (CMT4J) with a phenotype of rapidly progressive and asymmetric motor neuron degeneration, thus resembles motor neuron disease. A recent report also suggests heterozygous mutations of Fig4 are a genetic susceptibility factor for acquiring amyotrophic lateral sclerosis (ALS). Mice with homozygous loss of function mutation of the fig4 gene, called pale tremor (plt) mice, develop severe neuronopathy, and may serve as an animal model for investigating mechanisms of neurodegeneration. However, the pathological alterations in the plt CNS have not yet been well characterized. To investigate these issues, we performed an electron microscopic (EM) study on the mouse CNS. Cytoplasm of all cortical neurons and many glial cells in plt mice were filled with numerous high electron-dense granules that were reminiscent of lysosomes. Similar pathological changes were identifiable in about one third of the spinal anterior horn neurons. In contrast, this accumulation was not detectable in plt dorsal root ganglion (DRG) neurons and rarely visible in wild-type cells of CNS. Instead, plt DRG neurons contained excessive vacuoles that were single-membrane delimited and appeared electron transparent. Immunoreactivity against lysosomal proteins, NPC1 and LAMP2, was strong in many cells of plt brains, but hardly detectable in any wild-type cells. This was consistent with a robust increase of NPC1 and LAMP2 on Western blots, but not for mannose-6-phosphate receptor, an endosomal protein that is excluded from the lysosomes. In addition, cholesterol was also increased in plt cells. Taken together, our data suggest that Fig4 deficiency causes an abnormal lysosomal storage in the nervous system. We speculate that abnormal intracellular storage may be a pathogenic mechanism for neurodegeneration in the disease, and exert differential effect between spinal motor and sensory neurons. Supported by NINDS, MDA and Hiller ALS Foundation.

C64 DYSFUNCTION OF THE ENDOSOME PATHWAY INDUCED BY SOD1 MUTANTS

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Keywords: endosome, exosome, SOD1

Background: Secretion and extracellular action of SOD1 mutants is implicated in motor neuron degeneration in ALS, however the nature of the secretory pathway responsible remains unclear. ER-Golgi transport is proposed to mediate SOD1 secretion in culture, however evidence for SOD1 presence in the ER is conflicting at present. Endosomes represent one alternative export pathway and mediate secretion via exosomes originating from multivesicular bodies. The presence of SOD1 in the endosome compartment may therefore constitute another extracellular source.

Objectives: To investigate the contribution of the endosomal pathway to SOD1 secretion and relationship of endosomal function to cell pathology in familial ALS models.

Methods: NSC-34 cells stably expressing wild-type or mutant SOD1 were examined for expression of early and late endosomal markers using Western blotting and immunocytochemistry. Exosomes were purified by ultracentrifugation of cell conditioned medium and characterised by immunoblotting, sucrose density gradients and electron microscopy. Conditioned and exosome-depleted media were analysed as controls. Exosomes were also extracted and studied from cerebrospinal fluid from adult sheep as an animal model.

Results: SOD1 was identified in medium fractions enriched for the exosomal markers flotilin and TSG101, but not nuclear, mitochondrial or Golgi proteins. Isolated exosomes also conformed to predicted morphology, diameter and density. Exosomal SOD1 secretion was inducible by depolarisation and monensin treatment, while wortmannin blocked release in culture. In stable cell lines, wild-type SOD1 accumulated at high level in exosomes, while all mutants (dismutase active and inactive) were depleted from exosomes. Expression of late endosomal markers such as TSG101 was preserved in mutant cells, however early endosome proteins such as Rab5 were downregulated. Interestingly, analysis of exosome-depleted medium revealed presence of wild-type, but not mutant SOD1, suggesting a second pathway of secretion for normal SOD1. SOD1 was also identified in exosomes derived from ovine cerebrospinal fluid, confirming its exosomal secretion *in vivo*.

Conclusions: We demonstrate that SOD1 secretion is mediated by the endosome system which is disrupted by ALS-linked mutations, consistent with our previous findings of impaired mutant SOD1 secretion in a cellular model. The endosome pathway may therefore constitute a new possible therapeutic target for modulating extracellular SOD1 levels in ALS.

C65 LOSS OF RAC1-INDUCED MACROPINOSOMAL AND ENDOSOMAL LOCALIZATION OF ALS2 UNDERLIES THE PATHOGENESIS FOR MOTOR NEURON DISEASES CAUSED BY MISSENSE MUTATIONS IN THE ALS2 GENE

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Keywords: ALS2, missense mutation, endosomal localization

Background: Mutations in the *ALS2* gene account for a number of juvenile motor neuron diseases (MNDs), such as a juvenile recessive form of ALS (ALS2), a rare juvenile recessive form of primary lateral sclerosis (PLSJ), and an infantile-onset ascending hereditary spastic paralysis (IAHSP). Loss of function of its gene product ALS2/alsin accounts for neuronal dysfunction and degeneration. We and others have reported that ALS2 enhances endosome fusion by activating Rab5. Further, we have shown that ALS2 acts as a novel Rac1 effector, implicating a mode of endocytosis called macropinocytosis. Thus, ALS2 can mediate the Rac1-Rab5 signalling pathway, thereby regulating macropinocytosis and the following endosome trafficking and fusion. Recently, two missense mutations in the *ALS2* gene were identified. These mutations result in mutants ($ALS2^{C157Y}$ and $ALS2^{G540E}$) carrying a single amino-acid substitution in the regulator of chromosome condensation 1-like domain (RLD) located in the N-terminal region with the preservation of the C-terminal Rab5GEF domain. However, the molecular mechanisms by which these pathogenic mutants cause *ALS2*-linked MNDs are unclear.

Objectives: To elucidate the molecular mechanisms governing the functional loss of ALS2 due to a single amino-acid substitution in the RLD, we investigated molecular and cellular functions of these two pathogenic mutants.

Methods: Constructs expressing wild-type ($ALS2^{WT}$) and mutant ALS2 ($ALS2^{C157Y}$ and $ALS2^{G540E}$) were generated. Interactions between ALS2 mutants and either Rac1 or Rab5 were examined by *in vitro* GST pulldown assays. Rab5GEF activities of the pathogenic mutants were measured by *in vitro* [^{3}H]GDP dissociation assay on Rab5A. Homo-oligomerization of the mutant proteins was evaluated by co-immunoprecipitation experiments. Rac1-induced redistribution of the ALS2 mutants within the cells was assessed by immunocytochemical analysis.

Results: Similar to $ALS2^{WT}$, both $ALS2^{C157Y}$ and $ALS2^{G540E}$ interacted with Rac1 as well as with Rab5. Further, these mutants preserved the Rab5GEF activities *in vitro*, and the oligomerization capability, as does $ALS2^{WT}$. However, unlike $ALS2^{WT}$, these pathogenic mutants failed to localize to the Rac1-induced macropinosomes. These results indicate that while the ALS2 pathogenic mutants can still interact with Rac1, they lose their function as a Rac1 effector in cells. This could lead to the mislocalization of ALS2 within cells, and thus to loss of ALS2 function as a Rab5 activator on the macropinosome and endosome compartments.

Discussion and Conclusions: Our results indicate that the RLD of ALS2 is crucial to maintain the proper subcellular localization and function of ALS2. Thus, loss of Rac1-induced macropinosomal and the following endosomal localization of ALS2 might underlie the pathogenesis for MNDs caused by missense mutations in the *ALS2* gene. Future studies will provide insights not only into the fundamental role of ALS2 in macropinocytosis and the endosomal maturation in neurons but also into the pathogenesis underlying the *ALS2*-linked MNDs.

SESSION 8B COGNITIVE CHANGE

C66 FRONTOTEMPORAL DEMENTIA AND MOTOR NEURONE DISEASE

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Keywords: frontotemporal lobar degeneration, clinicopathological correlation, behaviour

An association between frontotemporal dementia (FTD) and motor neurone disease (MND) is now well recognised. Nevertheless, the precise nature of the relationship is not yet fully understood. It is not known whether people with FTD/MND are representative of the larger population of patients with forms of frontotemporal lobar degeneration (FTLD) or whether there are clinical, pathological and genetic differences. Similarly, it is unclear whether FTD/MND is a unique syndrome, clinically and aetiologically distinct from classical MND, traditionally thought to be a pure motor disorder, or whether there is a clinical continuum between the two.

In this talk I examine data from a cohort of more than 500 patients with clinical syndromes of frontotemporal lobar degeneration (FTLD) and compare the demographic, clinical and, where available, pathological characteristics of those individuals who develop MND and those who do not. The data reveal similarities between patients with and without MND in terms of age at onset and frequency of family history but highly significant differences in terms of gender ratio and illness duration. There are commonalities between patients with and without MND in cognitive/behavioural presentation, the majority showing the behavioural/executive disorder of FTD and a minority, features of semantic dementia or progressive nonfluent aphasia. However, in contrast to the pathological heterogeneity within FTLD, which encompasses tau and non-tau pathologies, and three sub-types of ubiquitin histopathology, the patients with MND show pathological homogeneity, sharing an identical tau-negative, ubiquitin positive histology. Molecular studies reveal no patient with MND to have mutations in the tau or progranulin gene.

Complementing these data, I describe the findings from a small-scale study of behaviour in MND. The findings point to a spectrum of behavioural change, with some patients exhibiting no behavioural alterations whereas others fulfil behavioural criteria for FTD.

The implications of these data for the "continuum" vs. "phenotypic variant" notion of the relationship between FTD and MND are discussed in the light of advances in molecular biology and genetics.

C67 EARLY VERBAL FLUENCY DEFICITS PREDICT COGNITIVE IMPAIRMENT IN ALS: A POPULATION-BASED LONGITUDINAL STUDY

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Keywords: cognitive, population, longitudinal

Background: Up to 60% of people with ALS have mild cognitive decline; a smaller proportion develop frontotemporal dementia. The population-based frequency, clinical characteristics and natural history of cognitive decline in ALS are unknown.

Objective: To determine the frequency and natural history of cognitive decline in ALS in a defined population-based cohort, using the latest consensus criteria (1).

Methods: All incident patients with ALS were captured on the Irish ALS Register and were asked to participate in a longitudinal study of cognitive function. Each participant was assessed using an extensive neurological and neuropsychological battery at 3 time intervals over 18 months.

Results: A population-based cohort of 87 patients and matched controls was studied. Clinical characteristics reflect the overall demography of ALS in Ireland. Twenty two (25%) had bulbar-onset disease. A total of 46 (53%) had deficits in verbal fluency on first assessment. Based on consensus criteria of 2 standard deviations below the control mean on two or more cognitive tests, 32.2% of ALS patients had cognitive impairment (ALSci) at time of first assessment. 7 (8%) patients were diagnosed with behavioural impairment (ALSbi+ as per supportive Neary criteria); 20.7%of patients were however behaviourally impaired as measured by a 2-standard deviation (SD) change from premorbid levels on either a FrSBe subscale or FrSBe total score. Apathy was the most marked behavioural change. Total behavioural change in patients predicted caregiver burden and mood.

17 (19%) patients met Neary criteria for FTLD: 12 for behavioural variant FTD, 2 for non-fluent progressive aphasia, and 3 for semantic dementia. Of those who tested within the normal range on the first assessment, only 3 (7%) had evidence of cognitive impairment on subsequent testing. Conversely, those who had verbal fluency deficits on first testing had clear evidence of deteriorating function on subsequent testing.

Conclusions: ALS is associated with a high prevalence of cognitive and behavioural change. Early deficits in verbal fluency predict later cognitive impairment. However, those with normal cognitive function at baseline are unlikely to exhibit any cognitive or behavioural deterioration. These data suggest that ALS with cognitive impairment represent a distinct subpopulation of ALS and not a continuum.

Reference:

1. Strong *et al.* Amyotrophic Lateral Sclerosis, 2009; 10(3):131–46

C68 VALIDITY OF A BRIEF COGNITIVE SCREENING EXAM FOR ALS PATIENTS

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Keywords: FTD, screening

Background: Recent investigations suggest that 28–48% of ALS patients possess a spectrum of frontotemporal deficits on neuropsychological measures, and patients with these cognitive and behavioral changes have poorer compliance and reduced survival rates. In this study, a brief screening battery was compared with a thorough neuropsychological evaluation to determine whether a time-efficient,

cost-effective screening exam can be a valid instrument in identifying ALS patients with cognitive and behavioral changes.

Objectives: This 30 minute cognitive screen is predicted to be a valid indicator of the cognitive and behavioral functioning of ALS patients. Specifically, diagnoses based on screening instruments are predicted to correlate with diagnoses based upon separate, standardized measures used in a full two hour neuropsychological battery. The diagnostic categories included ALS, ALS with Cognitive Impairment (ALSci), and ALS with Behavioral Impairment (ALSbi).

Methods: Based upon performance on a thirty minute screening battery, 14 study participants were classified into three diagnostic categories: ALS, ALSci, and ALSbi. Using a separate 2-hour neuropsychological evaluation, independent diagnoses were made. Comparisons were made between screening diagnoses and diagnoses made using standardized, age-matched norms. The screening diagnoses were based upon a cut off score of 12 or below on the ALS-Cognitive Behavioral Screen (ALS-CBS) and a score of 27 or above on the the Frontal Behavioral Inventory (FBI). Full battery diagnoses of ALSci were based on a 5^{th} percentile cut off on two or more measures of functioning. Full battery diagnoses of ALSbi were based on a T score of 65 or above on the Frontal Systems Behavioral Scale.

Results: In 12 of the 14 cases studied for cognitive functioning, matching diagnoses were made using the 30 minute battery. In two cases, the screening tests identified subjects as impaired but when given a full battery and requiring strict cut offs for pathology, they were diagnosed as normal. When measuring behavioral symptoms, 5 of the 7 subjects had matching diagnoses. In both cases, the screening test diagnosed the subject as normal but the full battery identified them as behaviorally impaired.

Discussion and Conclusions: These data suggest that the screening tool may be an effective instrument in identifying ALS patients with cognitive and behavioral changes, particularly when measuring cognitive changes. Behavioral changes may be more accurately measured with the full battery test (the FrSBe) as it was more sensitive than the Frontal Behavioral Inventory.

C69 VALIDATION OF THE PENN STATE BRIEF EXAM OF FRONTAL AND TEMPORAL DYSFUNCTION SYNDROMES IN AMYOTROPHIC LATERAL SCLEROSIS: APPLICATION OF GUILFORD'S STRUCTURE OF INTELLECT THEORY

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Keywords: frontotemporal dementia, assessment, treatment planning

Background: ALS-related cognitive behavioral impairments are estimated to occur in 30–50% of patients, primarily manifested as frontal and temporal dysfunction syndromes, with only a small percentage significant enough to meet the criteria for Frontotemporal Dementia. The Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes (PSFTS) is concise enough to be administered during the ALS multidisciplinary clinic visit, while sensitive enough to detect emerging declines in frontal and temporal cognitive behavioral capacities. Cognitive measures were chosen to detect declines in capacities recognized as important for decision making and problem solving. To account for the synergistic role of frontal and temporal cortical processing in decision making, we applied the Structure of Intellect Theory of Guilford during the selection of both brief exam measures and comprehensive neuropsychological measures to which we correlated brief exam findings. In Guilford's Structure of Intellect Theory, intelligence is viewed as comprising distinct Operations, Contents and Products. Operations include Evaluation, Convergent Production, Divergent Production, Memory and Cognition, the latter encompassing basic language skills. The Operation relevant to decision making is Evaluation, while Convergent and Divergent Production are Operations relevant to reasoning and problem solving.

Objectives: This study aimed to validate the PSFTS by correlation of findings to those obtained from a comprehensive neuropsychological battery of measures also constructed from Guilford's Operations, with an emphasis upon decision making and problem solving.

Methods: Thirty-five age, education and ALSFRS-R matched patients with an El Escorial diagnosis of probable ALS participated in the study. Non-parametric Spearman correlations were conducted between the brief exam and comprehensive exam measure findings classified by the Cognition, Evaluation, Convergent Production, and Divergent Production Operations.

Results: Statistically significant relationships were evidenced for all four Operations evaluated. Evaluation (decision making) relationships were evidenced for the brief exam task of Judgment and battery tasks of Consequences Obvious ($p < 0.014$) and Consequences Remote ($p = 0.054$). Problem Solving relationships included significance between 1) a brief exam task of Convergent Production (Similarities) and a battery task of Convergent Production (Missing Cartoons) ($p < 0.007$), as well as a battery task of Divergent Production (Alternate Uses), and 2) brief exam task of Divergent Production (letter fluency) and a battery task of Divergent Production (Alternate Uses) ($p < 0.0001$).

Discussion: Accounting for the synergistic role of frontal and temporal cortical processing in decision making by applying Guilford's Structure of Intellect Theory, we developed a brief exam applicable to the ALS multidisciplinary clinic, able to detect emerging declines in problem solving ability. Currently, we validated this brief exam by demonstrating the high degree of concordance between findings generated by 35 ALS subjects who completed both the brief exam and a comprehensive neuropsychological assessment, also selected by applying Guilford's Structure of Intellect Theory.

Conclusions: The PSFTS is a valid approach to early detection of frontal and temporal dysfunction syndromes by brief exam. The ability to identify emerging difficulties with decision making and problem solving in the ALS multidisciplinary clinic is of vital importance for optimal treatment planning.

C70 ANTI-SACCADE PARADIGM IN THE COGNITIVE FRONTAL ASSESSMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: anti-saccade paradigm, neuropsychological assessment, eye-movements

Background: Eye movement abnormalities are sensitive markers of neurological diseases and have been studied in a variety of neurological conditions (1–3). The analysis of saccadic eye movements may provide a useful tool for investigating neurological or psychiatric disorders in which the frontal lobe is impaired. Frontal alterations in Amyotrophic Lateral Sclerosis (ALS) have been variously described and assessed in literature (4). Involvement of frontal function has recently been studied exploring ocular fixation with the aid of eye-tracking technology, thus revealing the importance of detecting the whole spectrum of frontal involvement characterizing motor neurone disease's cognitive pattern (5). Anti-saccade paradigm is ideal in exploring frontal cognitive functions (6). In the anti-saccade paradigm subjects are instructed not to make a reflexive saccade to an appearing lateral target but to make an intentional saccade to the opposite side. This ability depends on the integrity of the dorsolateral prefrontal cortex (DLPFC) (7).

Objectives: The purpose of this study was to analyze frontal cognitive functioning of ALS patients with cognitive computerized measures and the anti-saccade paradigm.

Methods: Fifteen patients fullfilling El Escorial Criteria (8) for ALS and fifteen controls underwent an extensive neuropsychological and psychodiagnostic assessment. Patients received the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRSr). Different cognitive domains were investigated with both traditional cognitive tools as well as computerized tests: reaction time, attention, eye-movements, executive functions, cognitive flexibility, language, problem solving. This battery included: computerized measures of reaction time and eye-movements (PVA test-Visuo-Attentional Performance Evaluation), Frontal Assessment Battery (FAB), Stroop Colour-Word Test, Symbol digit modalities test, Trail Making Test, Mini Mental State Examination (MMSE), Raven's Coloured Progressive Matrices (CPM). Eye-movements were measured using a 1750 Tobii eye-tracker. Clinical tools for assessing psychological and emotional status included: MOS 36-Item Short-Form Health Survey (SF-36), Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory-Y (STAI-Y).

Results: Our data show quantitative and qualitative differences in cognitive performance between patients and controls, with higher difficulties in the anti-saccade task for the former and better scores in neuropsychological traditional tools for the latter. Significant differences and paradigmatic patterns of data emerge on different eye-movements/reaction times measures, while a general cognitive slowliness charcacterized patient's performances. Patients displayed lower performances on frontal measures of cognitive functioning. Subjects differed significantly for the presence of depressive/anxious symptoms.

Discussion and Conclusions: Neuropsychological assessment reveals specific cognitive and psychological patterns, as well as peculiar alterations of eye-movements patterns. Computerized neuropsychological assessment seem more sensitive in detecting small 'frontal' cognitive changes frequently observed at the onset of the disease. Moreover this assessment seems to be an ideal tool in assessing ALS longitudinally. These data suggest that oculomotor assessment and the anti-saccade paradigm may be useful in this kind of diagnosis.

SESSION 8C INTERNATIONAL PERSPECTIVES ON CARE MANAGEMENT

C71 PREDICTORS OF SOCIAL SERVICES INPUT AMONGST PEOPLE WITH ALS/MND

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Keywords: care, social services, predictive factors

Background: A descriptive audit of the key characteristics of the MND population in a large UK specialist MND clinic was undertaken in 2008 as part of a larger study exploring the personal experience of living with MND. The audit revealed that a significant number of patients and carers were not accessing external caring support provided by local social services, despite increased levels of disability (1).

Objectives: The aims of this study were to document changes in the key characteristics over a 12 month period in an attempt to identify factors predictive of uptake of social services care.

Methods: A comprehensive case note review of patients was undertaken at 2 time points, T1 (March 2008) and T2 (March 2009). Statistical analysis was aided by SPSS version 16.0.

Results: The sample had a mean age of 64.4 years (SD = 11.7 years), with a mean onset age of 59.1 years (SD = 12.6 years). Age at T1 and duration of the illness were both significantly correlated with ALS Health Severity Scale (ALSHSS) ratings. Only 27.1% of the sample were in receipt of local authority care at T1, with this rising to 37.7% of the surviving sample at T2. The presence or absence of local authority care at T1 was taken as a dependent variable in a logistic regression analysis employing onset age, time from diagnosis to T1, and ALSFRS scores as a predictive model. The model was highly predictive of the dependent variable, with each unit rise in ALSFRS scores being related to an 8.1% decrease in the odds of receiving local authority care at T1.
A separate logistic regression showed that forced vital capacity (% of predicted) at T1, and the time from disease onset to T1, were both positively predictive of being alive at T2. In particular, each unit increase in vital capacity at T1 was related to a 4% increase in the odds of being alive at T2.

Conclusions: This study has provided detailed information regarding the level of uptake of care services over a one year period for the current MND population followed up by a specialist UK MND clinic. It confirms previous findings of relatively poor uptake of care services by people with MND which may be predicted by taking into account age at onset, disease duration and ALSFRS scores. Additionally, there is confirmation of the role of forced vital capacity as a predictor of survival.

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C72 A CARING APPROACH TO ALS CARE

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Keywords: patient instruction, home care, quality of life

Background: Good patient care for ALS sufferers is a prerequisite for maintaining their quality of life. Knowledge about the disease and patient care is therefore essential for both patients and their caregivers. The patients' and caregivers' thirst for information was immense and could not be fulfilled in an adequate way during the regular outpatient consultations. To address this need, we designed a special training programme for ALS patients, caregivers and healthcare professionals.

Objectives: The aim was to enhance patients' and caregivers' knowledge about the disease and patient care and to enable adequate care for a tetraplegic person in a home setting.

Methods: We therefore offered five theme-based afternoons, each was a module lasting five hours. These took place in comfortable surroundings and included presentations from experts, question and answer sessions, practical group exercises under supervision and ample time to exchange experiences. The themes of the modules were "mobility", "breathing", "nutrition", "homecare" and "last phase". The ALS Care Training Programme is based on the adult education concept consisting of information, practical experiences with supervised training and the exchange of experiences. The programme was mainly designed for patients (P) and caregivers (CG) but health care professionals (HC) could also participate. A booklet for everyone to take home was handed out at each module.
Questionnaires with a self-rating scale of 0 to 5 (0 = no knowledge, 5 = best knowledge) were given out before and after each training session addressing the major goals of each theme.

Results: A total of 42 patients, 52 Caregivers and 21 healthcare professionals participated during the years 2005, 2006 and 2008. A total of 532 questionnaires were completed. Improvement of knowledge was noted for all themes. Average scores increased for mobility from 2.3 to 3.7 (P); 1.9 to 3.2 (CG); 3.5 to 4.0 (HC), for breathing from 2.5 to 4.3 (P); 2.3 to 4.1 (CG); 3.7 to 4.7 (HC), for nutrition from 3.1 to 4.5 (P); 2.8 to 4.5 (CG), for homecare from 2.9 to 4.3 (P); 2.4 to 3.9 (CG) and "last phase" from 2.1 to 4.3 (P); 2.3 to 4.3 (CG); 3.4 to 4.7 (HC). All improvements were highly significant ($p < 0.01$; paired t-test).

Conclusion: To the best of our knowledge, this is the first systematic ALS Care Training Programme to have been reported. This project shows that systematic ALS Care Training is a particularly effective and appropriate way of transferring knowledge to improve confidence in care management of patients and their caregivers. It could be an inspiration for others.
We would like to thank for their support: Fresenius Kabi, Switzerland, Aventis Sanofi, Switzerland, patients' sponsoring and since 2008 by the Swiss Muscle Society.

C73 ALS OUTREACH SERVICES

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Keywords: client satisfaction, education, outreach

Background: The Vancouver Coastal Health ALS Centre is British Columbia (BC)'s trans-disciplinary team providing services to people with Amyotrophic Lateral Sclerosis (ALS) from time of referral to end of life. The team is composed of clinicians in Neurology, Dietetics, Nursing, Occupational Therapy, Physiotherapy, Social Work and Speech-Language Pathology and offers outpatient diagnostic, assessment, intervention, consultation, outreach and educational services in partnership with other community organizations throughout the province. With the support of the ALS Society of BC, the ALS Centre developed a Mobile Outreach Clinic to better serve ALS clients, families and local service providers. This started as a pilot project and is now a permanent service offered by the ALS Centre. Approximately four clinics per year are offered to different regions of the province. Education sessions to local service providers were later added to the mobile clinic schedule to meet the learning needs of the community.

Objectives: 1) To evaluate the effectiveness of the Mobile Outreach Clinic and Education sessions offered to local communities. 2) To evaluate satisfaction levels of clients, families and local health care providers with the Mobile Clinic and Education session outreach model.

Methods: Satisfaction questionnaires including 5 items on a 5-point Likert scale and open-ended questions were distributed to clients seen during Mobile Clinics and to health care providers who attended the education sessions. Complete data were obtained from 34 clients and 55 health care providers. Results were collated and analyzed with descriptive statistics (crosstab and bar graphs) using statistical software (SPSS V15).

Results: ALS clients, their family and the local health care providers all seem to be highly satisfied by the outreach services they received from ALS Centre.

Discussion: The high level of satisfaction of the ALS clients and their local health care providers illustrates the relevance of outreach services, in particular the mobile clinics and the education of local professionals about the management of ALS symptoms. As a result, those services have now been established as part of the permanent service provision offered by the ALS Centre.

Conclusions: The mobile outreach clinic project demonstrated high satisfaction from clients living in various regions of the province of British Columbia. The local health care providers involved with ALS clients expressed high satisfaction about education provided by the ALS Centre. However, this model of service provision does not serve some of the ALS clients living away from major urban centres: for example, clients living in facilities or home bound with limited transportation options. Future options to consider may include: home visits to facilities and client homes in rural areas, extending mobile clinic operating hours, and videoconference education sessions.

C74 OUTCOMES USING AN ON-LINE PRE-CLINIC ASSESSMENT TOOL

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Keywords: pre-clinic assessment, communication, satisfaction

Background: Multidisciplinary ALS clinics use pre-clinic assessments to gather information before the patient is seen by each team member. Typically, these are sent by mail or completed over the telephone. Telephoning patients can be time consuming and, over time, disease progression can impact the patient's ability to communicate verbally.

Objectives: To develop an on-line survey that can be completed by the patient prior to their multidisciplinary visit. Secondary objectives were: to improve efficiency in obtaining information; to provide current information to the team members so that they can be prepared with equipment, teaching tools, information etc. to meet the needs of our patients; to prioritize the issues to be addressed by the team and to streamline the clinic process.

Method: Using Survey Monkey ®, a survey was developed by polling the team members for questions and concerns covered during the clinic visit. The survey included questions regarding change in medical status, medications, swallowing, nutrition, breathing, speech, mobility, medical equipment and family concerns (disability, wills, power of attorney). Patients were sent the survey link by email one week before their visit. Patients without email were called and the survey was completed by one of the team members. The survey link also served as a reminder of the upcoming visit. Patients with incomplete surveys were contacted to determine if a phone interview was needed. Surveys went to a secure server monitored by one of the clinic RNs. Identified concerns or questions needing immediate attention were addressed as indicated. Two days before clinic, responses were forwarded to all team members. Following the clinic visit, a questionnaire was sent to the patients who completed the on-line survey, asking for feedback on the process and suggestions for improvement. Revisions were incorporated into the survey.

Results: All patients but two (2) who attended the clinic between December 2008 and April 2009 completed the survey either by email (78%) or with a team member via telephone (20%). Of the 67% of patients who completed the follow up survey, 78% perceived their quality of care at clinic was improved by completing the pre-clinic survey and 52% perceived their visit took less time and clinic flow was improved. A follow up survey with team members indicated they felt more prepared to answer questions and provide appropriate interventions. Loan Bank equipment utilization was improved with less need for home delivery, impacting time and travel costs. Time spent by team members on the telephone (pre and post clinic) was cut by more than 50%.

Conclusions: On-line pre-clinic assessment surveys can be a beneficial tool to help multidisciplinary teams prepare for and respond to patient needs.

C75 ENHANCING SPEECH DURING BIPAP USE

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Keywords: BiPAP, speech, quality of life

Background: The efficacy and benefit of BiPAP use in amyotrophic lateral sclerosis (ALS) is well established with daily utilization varying from several hours to 24 hours depending on disease progression and stage. Problems associated with BiPAP use include dry mouth, abrasion from the mask and claustrophobia. These can be dealt with relatively easily. A more major problem is its interference with speech caused by muffling of vocalization by the BiPAP mask and the associated airflow noise. For patients using BiPAP several hours a day this problem greatly limits their communication.

Objectives: To improve speech when using Bi-level Positive Airway Pressure (BiPAP).

Methods: To overcome muffled speech due to BiPAP use a small electret microphone was suspended near the top of the mask away from the direct path of the rushing air. The sound output was filtered and amplified by software on a digital signal processing board that computes an average of the noise during non-speech activity and then removes it from subsequent sound frames. Residual noise in a sound frame is then further attenuated based on information provided by an algorithm that examines adjacent sound frames.

Results: During preliminary testing, it was found that the airflow noise was removed almost entirely with very little residual noise from the sound signal and after amplification, allowing listeners to hear a BiPAP user's voice very clearly. Occasionally nasal bridge prominence caused the microphone to be flush against the skin and speech capture was impossible. Placing the microphone nearer the mouth overcame this problem but the airflow noise increased significantly affecting the performance of the noise filtering. Possible solutions involve finding an alternative optimal placement in the mask or designing a microphone housing that would help shield the microphone from noise. Sometimes reverberations in the mask resulted in a "radio-like" speech; a possible solution to this would be use of multiple microphones.

Discussion and Conclusions: The prototype developed allows significant improvement in speech whilst using a BiPAP. We believe that this is the first attempt to solve the problem of audible speech whilst using a BiPAP. Several modifications are being tested to improve the aesthetics and other aspects of the present prototype and we are presently evaluating its use in a larger cohort of ALS patients.

C76 THE SURVEY REPORT OF THE ALS PHYSICIANS IN JAPAN

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Keywords: palliative care, withdrawal of permanent ventilation, questionnaire

In March 2009 we sent out a questionnaire to the 4,500 Board Certified Membership of the Japanese Society of Neurology to ask 30 questions about their clinical experiences, practice and thoughts in connection with palliative care at the terminal stage and patients' rights, especially about the withdrawal of permanent ventilation. This is the very first of this kind of comprehensive survey in Japan to cover the exclusive population of ALS physicians. By the end of April, 1,470 anonymous responses (33%) had been returned. To date, 700 responses have been analyzed.

The purpose of the survey is to grasp what is happening to the physician's side of the Japanese ALS community where the high rate of ventilated ALS patients and the low rate of morphine prescription for ALS patients have often been pointed out.

As for the palliative care practice, 21% of the respondents prescribe morphine, which shows a drastic increase from the 14% in the 2007 survey. However, 72% of them had less than 5 patients. 50% of them studied and trained themselves when they began prescribing morphine. It illustrates that most of the neurologists are not well experienced with morphine, and that they are isolated in their practice. This may be explained by the fact that the Japanese national medical insurance does not pay for long acting morphine administered to ALS patients. However, 45% of the respondents answer that they would prescribe morphine whether or not the national insurance pays. This implies that the physicians' perspective about morphine prescription in the clinical setting is changing. On the other hand, approximately 30% of the respondents identify morphine prescription with euthanasia.

As for the withdrawal of permanent ventilation, which is not legal in Japan, 21% of the respondents were asked by their patients to turn off the ventilation, and 6% of them agreed and accommodated the patient's wish. 77% of them declined and explained they were not allowed to do so. 18% of them declined and explained they were not supposed to do so. While 23% of the respondents believe that no discussion of whether the withdrawal right is permitted or not should be promoted, 46% believe that such rights should be granted if the decision made by the patient and/or his/her family members can explicitly be recognized. The results illustrate that the physicians are also divided.

It is worth noting that 20% of the respondents commented on their own, aside from answering the questionnaire, to share their thoughts. It may be the time to lay the foundation for the Japanese ALS physicians to discuss openly and candidly together to deal with the wants and wishes of their patients.

SESSION 9A INFLAMMATORY/ IMMUNOLOGICAL AND GLIAL CONTRIBUTION TO MOTOR NEURON DEGENERATION

C77 NEUROINFLAMMATION – CAUSE AND CONSEQUENCE FOR DEGENERATIVE DISORDERS

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Keywords: neuroinflammation, cytokine, microglia

Activation of the innate immune system either by external stimuli or by brain intrinsic mechanisms including aggregated, misfolded or post-translationally modified peptides and proteins has direct consequences for bystander cells including astrocytes and neurons. While generation of cytokines and chemokines in an acute phase may not necessarily be detrimental, the chronic exposure of glia and neurons to neuroinflammatory mediators causes dysfunction and degeneration. Recent evidence indicates that all major neurodegenerative disorders, including Alzheimer's disease, Amytrophic Lateral Sclerosis and Parkinson's disease are characterized by an inflammatory component, in addition to their respective classical pathological changes. The presence of inflammatory mediators alone, is able to affect key neuronal functions such as hippocampal long-term potentiation, an integral mechanism of memory consolidation or axonal transport of synaptic vesicle precursors. Sustained exposure of glial cells in turn, reduces their trophic support for neighbouring neurons and the uptake of glutamate from the synaptic cleft. While it remains unclear whether inflammation is a primary cause, increasing evidence suggests that inflammatory mechanism interact with neurodegenerative pathways such as excitotoxicity, protein aggregation, APP processing and several others. Chronic neuroinflammation also compromises the neuronal antioxidative defense mechanisms thereby potentiating neurodegenerative events. Together this indicates that neuroinflammation, once stimulated may act as a motor for ongoing neuro- and glial dysfunction and death. New developments of PET tracers now allow for a direct assessment and monitoring of microglial activation in patients. Epidemiological data suggest a beneficial effect of anti-inflammatory treatment strategies at least for Alzheimer's and Parkinson's disease. Therefore, future therapies, when initiated within the time window of opportunity, should also successfully target the inflammatory component of neurodegenerative disease.
The presentation will give an overview of the mechanisms concerned and present results from cell culture experimentation, animal studies to human epidemiology and clinical studies, highlighting in particular the role in and for motor neuron disease.

C78 INNATE AND ADAPTIVE IMMUNITY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): EVIDENCE OF COMPLEMENT ACTIVATION

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Keywords: innate immunity, complement, inflammatory cells

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by loss of motor neurons and gliosis in motor cortex (MCx) and spinal cord (SC). Although the etiology of ALS is still unclear, recent evidence suggests a role for the immune system in the disease process.

Objectives: To analyze the presence and distribution of inflammatory cells, such as microglia/macrophages and T lymphocytes as well as components of the complement system and their expression in sporadic and familial ALS cases.

Methods: We investigated the distribution of cellular components of innate and adaptive immunity using immunohistochemistry on SC and MCx sections of both sporadic (n = 16; sALS) and familial (n = 4; with frontotemporal dementia; FTD-ALS) ALS cases. We analyzed the expression and cellular distribution of complement components on both protein and mRNA levels as well. Quantification was performed for all stainings.

Results: In all ALS cases a prominent presence of microglial cells expressing class II-antigens (HLA-DR) and CD68-positive macrophages was found in both SC (ventral horn and corticospinal tracts) and MCx. We also observed perivascular and parenchymal T-lymphocytes ($CD3^+$; with a predominance of $CD8^+$ T-cytotoxic/suppressor cells) and the presence of dendritic cells (DCs; DC-$SIGN^+$). Quantitative analysis showed a significantly higher number of HLA-DR^+, $CD68^+$, $CD3^+$, $CD8^+$ cells and DCs in ALS SC and MCx compared to control tissues. The number of microglia/macrophages and T-lymphocytes was higher in long term sALS patients as compared to sALS patients with rapid ALS progression. In contrast DCs were more prominently observed in patients who had a more rapid progression. Several components of the complement cascade (C1q, C3c, C3d and MAC) were observed in active microglia and reactive astrocytes in SC and MCx of ALS patients.

Conclusions: Our findings demonstrate a persistent activation of immune/inflammatory responses in ALS, including the activation of the complement system. Understanding the role of complement activation in motor neuron degeneration in ALS may be of great importance in the development of new therapeutic strategies.

C79 CD4+CD25+FOXP3+ REGULATORY T CELLS INFILTRATION INDUCES ALTERNATIVELY ACTIVATED M2 MICROGLIA AND A STABLE PHASE OF DISEASE IN THE MSOD1G93A MODEL OF ALS

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Keywords: CD4+CD25+ T-cells, microglia, alternatively activated

Background: Current evidence suggests that motoneuron injury in ALS and mSOD1 mice is non-cell autonomous and involves microglia and infiltrating immune cells. While previous data indicate that microglia play a pivotal role in rate of disease progression, recent evidence indicates that CD4+ T-cells also have a critical function modulating microglial activation and slowing motoneuron degeneration. A lack of functional CD4+ T-cells in mSOD1/RAG2$^{-/-}$ and mSOD1/CD4$^{-/-}$ mice resulted in attenuated microglial morphological activation, yet increased pro-inflammatory and cytotoxic factors, reduced anti-inflammatory and neurotrophic factors and glial glutamate transporters, and decreased survival due to the absence of a stable phase of disease. The stable phase of disease is not specific to the G93A mutation in SOD1; a similar phase has been described between onset and a rapid progression phase in several ALS mouse models with different SOD1 mutations.

Objectives: To investigate which sub-populations of CD4+ T cells could be responsible for inducing the stable phase of disease and examine whether alternatively activated (M2) vs. classically activated (M1) microglial phenotypes contribute to the stable and rapidly progressing phases of disease in mSOD1^{G93A} mice.

Methods: The sub-populations of T lymphocytes in the blood, lymph nodes, and spinal cord of mSOD1 mice were analyzed during critical time points based on disease progression by flow cytometry and QRT-PCR. Evaluations were also made of M2 markers, anti-inflammatory cytokines, neurotrophic factors and of M1 markers, reactive oxygen species generating enzymes, and inflammatory cytokines over time from spinal cord by QRT-PCR and flow cytometry.

Results: In mSOD1 blood, there were increased CD4+ CD25+, CD4+FoxP3+, and CD25+FoxP3 T lymphocytes at 11 and 14 weeks of age. End-stage mice contained less CD25+FoxP3 T lymphocytes with no change in CD4+ CD25+ T cells. In lymph nodes, the increase in CD4+ CD25+, CD4+FoxP3+, and CD25+FoxP3+ T lymphocytes were not observed until 16 weeks of age. CD3+ and CD4+ were increased at end-stage disease in mSOD1 mice. Evaluations of microglial phenotypes during the stable phase of disease reveal that microglia are converted to a neuroprotective M2 phenotype, expressing increased levels of Ym1, BDNF, and TNFa. However, during the rapid progression phase, the balance is shifted toward an M1 phenotype, expressing reduced levels of Ym1 and BDNF and increased levels of NOX2, IL-1β, and IFNγ. Without the presence of T cells, the balance is shifted more toward an M1 phenotype, expressing even more NOX2.

Discussion and Conclusions: These results reveal an expansion of CD4+CD25+FoxP3+ regulatory T cells which infiltrate the spinal cord and modulate the M1/M2 balance of microglia. These data provide further evidence that disease progression in ALS is non-cell-autonomous and is modulated by the innate and the adaptive immune system. These glial/T-cell interactions establish novel targets for therapeutic intervention and validate immunomodulatory therapies in ALS.

C80 ANTI-CD40L MONOCLONAL ANTIBODY THERAPY IMPROVES DISEASE PROGRESSION AND SURVIVAL IN A MURINE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1_G93A, immunomodulation, biological

Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by skeletal muscle atrophy, weakness, and ultimately total paralysis. The pathology results from an unremitting degeneration of motor neurons in the spinal column and brain. As of yet, there is no therapy that improves patient survival.

The precise mechanism of disease onset and progression remains unclear; however there is an humoral immune response prior to the onset of severe clinical symptoms. Microgliosis, astrocytosis and infiltrating inflammatory cells from the periphery into the central nervous system have been well described in ALS patients and in transgenic mouse models, such as SOD1-G93A mice. 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 (1,2).

In an effort to extend on these findings, we have focused on modulating peripheral T cell activity using an antibody known to impact the T cell co-stimulatory pathway. A large body of work has established that blocking antibodies to CD40L ameliorate disease progression and survival in a variety of preclinical models of chronic inflammation including; experimental allergic encephalomyelitis (EAE) a model of multiple sclerosis, collagen induced arthritis, and systemic lupus erythematosus (3).

Here we report on a genome wide expression profiling study that has identified presymptomatic upregulation of the co-stimulatory pathway in the spinal cord, skeletal muscle, and sciatic nerve of SOD1-G93A mice. Based on these observations we hypothesized that systemic administration of anti-CD40L antibody would slow the onset and progression of disease in the SOD1-G93A mouse. Highly powered (n = 18 mice/group/gender), efficacy studies using optimized dosing protocols demonstrate that weekly administration of anti-CD40L antibody improves body weight and significantly slows the progression of paresis relative to age matched, gender balanced, litter mate controls. Furthermore, anti-CD40L antibody administration improves survival (median survival increase = 13 ± 0.3 days in treated versus controls; 141d vs. 128 d; $p < 0.007$; hazard ratio: 0.35 ± 0.14) in this ALS preclinical murine model. Data will be presented showing that these therapeutic effects appear to be mediated by modulation of the immune response in the peripheral nervous system. Finally, this work raises the possibility that immunomodulatory biologics, with proven efficacy in other

indications, could hold promise as a therapeutic strategy for ALS.

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C81 ASTROCYTIC MCT1 CONTRIBUTES TO MOTONEURON DEGENERATION IN ALS

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Keywords: motoneuron, astrocyte, viral vector

In Amyotrophic Lateral Sclerosis, a fatal neurodegenerative disease characterized by progressive loss of spinal and cortical motor neurons, astrocytes are proposed to play a significant role in disease pathogenesis. Astrocytes support neuronal function by secreting trophic factors, scavenging glutamate from the synapse, and providing metabolic support in the form of lactate. In this study, we were interested in examining the supply of lactate from astrocytes to motoneurons in ALS patients, and the mutant $SOD1^{G93A}$ transgenic mouse model of ALS. We identified a selective loss in the expression of the astrocytic lactate transporter MCT1 in the spinal cord of both sporadic and familial ALS patients. Reduced astrocytic MCT1 expression was also evident in mutant $SOD1^{G93A}$ transgenic mice. The effect of reduced astrocytic MCT1 expression on neuronal viability was examined both *in vitro* and *in vivo*. *In vitro*, reduced MCT1 expression in astrocyte-motoneuron co-cultures significantly reduced neuronal viability, while *in vivo* crossbreeding MCT1 heterozygous knock-out mice with mutant $SOD1^{G93A}$ transgenic mice resulted in an earlier onset of disease. The therapeutic effect of increasing MCT1 expression in mutant $SOD1^{G93A}$ transgenic mice was examined using recombinant adeno-associated viral vectors, where intraspinal injection of AAV2/6:MCT1 had significant neuroprotective effects. Combined, these findings demonstrate that astrocytic MCT1 is required for neuronal survival and contributes to motoneuron degeneration in ALS.

SESSION 9B EXERCISE, METABOLISM AND NUTRITION

C82 IS EXERCISE A PREDISPOSING FACTOR IN ALS? THE CASE FOR

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Keywords: exercise, physical activity, risk

ALS is a neurodegenerative disorder of the adult life. The cause of ALS in sporadic cases is still unknown. Among the possible causes, physical exercise, including sport activity, has been considered a predisposing factor. From the epidemiological point of view, several case control and cohort studies have evaluated the relationship between ALS and physical activity/sport participation. Most of these studies demonstrated an increased risk for heavy physical activity, with an odds ratio ranging from 1.5 to 2. While these data tend to support some effect of physical activity on ALS risk, these studies have several pitfalls, including the selection of cases and controls, the different operative definition of physical activity and the possible effect of confounding factors. According to one study (1) physical activity may be associated with an earlier age at onset of ALS. Cohort studies on professional athletes (soccer, bicycle riding, American football, basketball) did not demonstrate any specific effect of professional sport activity in ALS, since only professional soccer has been related with ALS (2,3). However, in this study the young age of onset of ALS in soccer players could indicate an effect of anticipation of ALS presentation related to strenuous physical activity. Physical activity could increase motor neuron degeneration because of stimulation of motor neurons in a pre-existent excitotoxic environment. Furthermore, physical activity may lead to increased motor neuron death through an unbalance between free radical formation and radical scavenger systems, with a consequent increased oxidative stress.

Data on the pre-clinical models of ALS are intriguing. In a study comparing $SOD1^{G93A}$ mice under enriched vs. standard conditions better motor performances were observed in the preclinical phase, but an early age at onset of symptoms and a lower survival was observed in female mice (4). This finding has been explained by increased motor neuron firing rates and activity induced by environmental enrichment that may accelerate excitotoxicity. In another study, using the same animal model, male endurance-trained mice had an hastened death compared to sedentary mice (5). Conversely, a study (6) showed that regular exercise significantly increased the life span in $SOD1^{G93A}$ male mice. Therefore, we have no unequivocal indication from the preclinical model of ALS on the effect of exercise on the onset and the course of neurodegeneration.

Data on physical activity and ALS are still too sparse and contradictory to be able to give a firm conclusion. Future epidemiological studies, looking at physical activity with a sound design, as well studies on the possible biological effect of physical activity on motor neurons are warranted.

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C83 IS EXERCISE A PREDISPOSING FACTOR FOR ALS? THE CASE AGAINST

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Keywords: exercise, risk factor

The notion that exercise could be a risk factor for developing ALS may have been supported by the case of Lou Gehrig, the famous American baseball player with ALS who dramatically became manifest during a match when failing to hit the ball. A relationship between exercise and ALS has been suggested by Italian investigators who did a retrospective study of the occurrence of ALS in all Italian professional football players from the series A and B in the period between 1970 and 2001. Risk of developing ALS was higher (1). In a prospective extended study of the original cohort they identified 2 new cases. Mean onset was almost 20 years lower compared with population studies (2). Interestingly, they identified no ALS cases in large control cohorts of professional road cyclists and basketball players. Whether a relationship between exercise and ALS exists, cannot be concluded from these studies. We found no relationship between lifetime physical activity and sporadic ALS (3). However, increased leisure time activities were associated with earlier age at onset. This suggests that premorbid leisure time activities in a population at risk of developing ALS - for other reasons than physical activity – could accelerate onset of ALS.

Experimental studies in hSOD1 mice have not been very helpful to answer this question either (4). Exercise may even be beneficial in terms of survival (4,5). A small clinical trial of resistance exercise showed that patients who trained had higher ALSFRS and SF-36 scores (6). A beneficial effect of training has been demonstrated in other neuromuscular diseases, e.g. Guillain-Barré syndrome, myasthenia gravis and some muscular dystrophies (7). Psychological factors may play a role. Two Cochrane reviews failed to demonstrate a beneficial effect of exercise programmes on functional ability in patients with peripheral neuropathy or muscle disease (8,9). There were no adverse effects. Aerobic training was not well tolerated in patients with Kennedy disease (10).

ALS is considered a multifactorial disease with identified and unidentified risk factors. Identified risk factors include the male sex, smoking, and genetic factors. The next 5 years will reveal what other genetic factors are implicated and what functions these genes have. Excitotoxicity from various causes may induce neuronal death. Mitochondrial dysfunction may be a final common pathway. Disturbance of axonal transport can be a second mechanism. When reinnervation fails, skeletal muscle weakness in ALS results from a shift towards denervation of muscle fibres. The insulin-growth factor I

could act at the level of the muscle fibre or on axonal outgrowth.
Prolonged neuronal excitation in epilepsy can lead to post-ictal paresis, but not to permanent deficit in itself. In conclusion, exercise cannot be held responsible for development of ALS.

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C84 HYPERMETABOLISM IN ALS: IMPLICATIONS FOR NUTRITIONAL MANAGEMENT

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ALS is increasingly recognized as a multi-system disorder. Beyond neurodegeneration, ALS patients display striking alterations of their energy homeostasis, including increased energy expenditure (hypermetabolism) and hyperlipidemia (1–5). Such abnormalities are also found in ALS animal models (6, 7), suggesting that they are intrinsic to the disease process. Most importantly, increasing energy intake of mutant SOD1 mice mitigated their symptoms (7) and increased lipemia positively correlated with survival in a cohort of 385 ALS patients (5). The importance of these findings for defining potential therapeutic strategies and improving nutritional management of ALS patients will be discussed, along with new results showing that muscle hypermetabolism is *per se* sufficient to drive motor neuron degeneration (8).

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C85 PROGNOSTIC SIGNIFICANCE OF NUTRITIONAL PARAMETERS IN ALS PATIENTS

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Keywords: bioimpedance, phase angle, hydration

Background: Malnutrition has been shown to be associated with a poor survival of ALS patients. Malnutrition can be easily evaluated with % of weight loss or body mass index. Other more sophisticated methods have been used to assess the nutritional status of ALS patients. Of those, total body impedance analysis has been validated to assess lean mass and evaluate phase angle, a marker reflecting the nutritional status and the cellular membrane alteration.

Objective: Our aim was to assess the independently prognostic significance of nutritional parameters in survival of patients suffering from ALS.

Methods: Our study included all patients with available nutritional evaluation during their follow up in our referral center located in France. Evaluated variables were body mass index, weight, tricipital skin-fold thickness, midarm muscular circumference, phase angle, lean mass, hydration disorders (extracellular to intracellular water ratio (E/I)). We used the Cox proportional hazard model to perform a survival analysis from time of diagnosis until death or censoring time. We considered variables collected at first evaluation as fixed variables and during the entire follow up as time varying covariates. Our analyses were adjusted on sociodemographic characteristics and neurological clinical status at baseline.

Results: Among 175 patients with at least one nutritional evaluation, we identified that an increase in phase angle was significantly and independently associated with a better survival and that hydration disorders (extracellular to intracellular water ratio (E/I) increment) were significantly associated with a shorter survival.

Discussion: The identification of new prognostic factors of ALS may be useful for clinicians to monitor nutritional status of ALS patients and for researchers to emphasize new hypotheses on the physiopathology of the disease.

C86 DIET AT TIME OF PROCEDURE PREDICTS COMPLICATIONS AFTER PERCUTANEOUS ENDOSCOPIC GASTROSTOMY PLACEMENT IN ALS

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Keywords: percutaneous endoscopic gastrostomy, dysphagia, outcomes

Background: The current ALS practice parameters of the American Academy of Neurology state that PEG is indicated for patients with ALS who have symptomatic dysphagia and should be considered soon after symptom onset. These parameters state that PEG should be placed while the patient's vital capacity is still above 50% of predicted in order to ensure optimal safety and efficacy.

Objectives: The goal of this retrospective study was to assess predictors of outcome and survival after PEG placement at a single center.

Methods: Patients with probable or definite ALS seen between January 2006 and April 2009 were identified by chart review. Forced vital capacity (FVC), ALS Functional Rating Scale score, diet and BMI were captured at time of diagnosis and at time of procedure. The primary outcome measure considered was the presence of any complication (pulmonary, PEG-related, prolonged hospital stay, tracheostomy, and/or death).

Results: Fifty-eight patients (29 female, 29 male) had PEG tube placement. The mean age of patients was 56 ± 13.3 years. Of these, 16 (27.6%) patients had bulbar onset of disease. Indication for PEG tube placement was weight loss in 39 (67%) patients, FVC $<50\%$ of predicted in 11 (19%), and elective placement in 8 (14%). Diet at time of PEG tube placement was regular in 18 (33%) patients, dysphagia level 3 in 24 (44%), dysphagia level 2 in 6 (11%), dysphagia level 1 in 1 (2%), and NPO in 5 (9%). The rate of decline in BMI was 0.2 ± 0.5 units per month (median 0.2, range 1.2 lost to 2.3 gained). The best predictor of complications from PEG placement in our patient sample was the consistency of diet at time of PEG. The more impaired the diet, the higher the likelihood of having at least one complication ($p = 0.02$), and pulmonary complications in particular ($p = 0.005$). The odds ratio for perioperative complications with significant modification in diet (dysphagia level 2, dysphagia level 1 or NPO) was 7.9 (CI: 1.9 to 33.4; $p = 0.005$) and increased after controlling for indication for PEG placement. FVC at time of diagnosis or time of PEG placement or impaired FVC ($<50\%$) was not predictive of perioperative complications or survival ($p > 0.40$). Six month survival was 69% and was correlated with total weight loss between diagnosis of ALS and PEG placement ($p = 0.03$) and rate of weight loss ($p = 0.02$), as well as a slower rate of disease progression ($p = 0.05$) and the absence of any complications ($p = 0.03$).

Conclusions: In our sample, consistency of diet was a more reliable predictor of complications after PEG placement than reduced FVC and may be a good proxy for airway clearance. Careful monitoring of weight and placement of PEG prior to significant adjustment in diet may help prevent perioperative complications and improve 6 month survival.

SESSION 10A AXONAL TRANSPORT AND MAINTENANCE

C87 ANALYSIS OF AXONAL TRANSPORT: FROM *IN VITRO* SCREENS TO REAL TIME *IN VIVO* ASSAYS

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Keywords: axonal transport, siRNA screen, SOD1

Axonal transport is essential for the maintenance of neuronal function and deficits in transport are known to result in neuronal degeneration in several areas of the nervous systems. Evidence from animal models of Amyotrophic Lateral Sclerosis (ALS) suggests that axonal transport deficits may contribute to pathogenesis, although our understanding of how these relate to disease progression remains unclear.

A major focus of our laboratory is to understand the machinery controlling sorting and long-range axonal transport in motor and sensory neurons. To fulfil this task, we have extensively exploited the high affinity and specific traffic of the binding fragment of tetanus toxin (TeNT H_C) and more recently those of some neurotrophic viruses. TeNT H_C enters motor neurons at the neuromuscular junction and is targeted to the spinal cord in axonal carriers shared with neurotrophins and their receptors Trks and $p75^{NTR}$. This route requires a specific subset of small GTPases and relies on Rab7 activity for long-range axonal transport powered by cytoplasmic dynein.

To identify novel players in this transport mechanism, we have performed a siRNA screen in motor neurons derived from mouse embryonic stem (ES) cells using a library of siRNAs directed against genes involved in endocytosis and membrane traffic. A high-throughput transfection method was optimised and used to perform the screen using the endocytosis of TeNT H_C and an antibody directed against $p75^{NTR}$ as a readout. Positive and negative regulators of TeNT H_C and $p75^{NTR}$ trafficking were identified. Candidates were validated using independent siRNA pools and secondary screens implemented on selected regulators of cytoplasmic dynein. Hence, siRNA approaches using differentiated ES cells represent a powerful tool to investigate traffic events in neurons and for the discovery of novel players in this biological process.

To extend our understanding of the regulation of axonal transport *in vivo* and its alteration during disease, we have established in parallel a dynamic *in vivo* assay that allows us to monitor retrograde transport in the intact sciatic nerve in anaesthetised mice**.** This assay permits the quantitative analysis of physiological axonal transport in wild type (WT) mice *in vivo* and its changes in $SOD1^{G93A}$ animals at different disease stages. Analysis of $SOD1^{G93A}$ mice revealed significant alterations in retrograde transport of TeNT H_C from a presymptomatic stage. This presymptomatic stage appeared to represent the point of onset of retrograde transport deficits in this MND model.

The results of this study reinforce previous evidence that indicates altered axonal transport in ALS and provides a new method to closely examine and potentially dissect out the mechanisms underlying axonal transport defects in mouse models of ALS.

C88 AXONAL RNA TRANSPORT: ROLES IN MOTOR NEURON FUNCTION AND DEGENERATION

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Keywords: axonal RNA transport, spinal muscular atrophy, active zones

Human motoneuron diseases are characterized by loss of motor endplates, axonal degeneration and cell death of motoneurons. The identification of responsible gene defects for familial ALS, spinal muscular atrophy (SMA) and spinal muscular atrophy with respiratory distress (SMARD) has pointed to distinct pathophysiological mechanisms responsible for the various forms of the disease. Evidence from mouse models suggests that enhanced vulnerability and sensitivity to proapoptotic stimuli is only responsible for some but not all forms of motoneuron disease (1–3). Reduced levels of the survival motoneuron (SMN) protein, which are responsible for SMA lead to disturbed RNA processing in motoneurons. A prominent phenotype of SMN deficient motoneurons is reduced axon elongation in the absence of defects that result in reduced motoneuron survival (4). In particular, the axonal transport of the mRNA for b-actin is severely reduced. The SMN protein is part of a complex in the cell body that assembles U snRNP particles. These U snRNP particles are central constituents of the spliceosome. In addition, the SMN protein is part of another complex in axons and axon terminals of motoneurons. This complex is distinct from the classical SMN complex, and it includes mRNA transport proteins, in particular the hnRNP-R protein (5). The hnRNP-R protein binds directly to b-actin mRNA, and both cell culture experiments in which hnRNP-R expression is reduced and *in vivo* studies point to an essential role of SMN/hnRNP-R interaction for axonal translocation of b-actin mRNA.

The consequence of reduced SMN levels in motoneurons is a severe depletion of b-actin-protein in axon terminals, resulting in disturbed axon elongation, reduced growth cone size and functional deficits in neurotransmission that are caused by disturbed integration and clustering of voltage-gated calcium channels in axon terminals (6). The deficit in clustering of voltage-gated calcium channel in growth cones of SMN-deficient motoneurons is accompanied by a significant reduction of spontaneous Ca^{2+} transient frequency. Current research in our lab focuses on the development of imaging techniques to visualize local translation of mRNAs in axon terminals of cultured motoneurons, and to develop techniques that allow biochemical analysis and characterization of mRNA transport complexes in isolated motoneurons. In addition, new mouse models are generated that allow analysis of axon abnormalities and disturbed synaptic function at the neuromuscular endplate *in vivo* by multiphoton microscopy.

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C89 DOWNREGULATION OF GENES WITH A FUNCTION IN NEURITE OUTGROWTH AND SYNAPSE FORMATION IN MOTOR NEURONES OF THE VEGF$^{\delta/\delta}$ MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Vascular endothelial growth factor, microarray analysis, neurite outgrowth

Background: Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen that stimulates vasculogenesis. It has also been shown to act as a neurotrophic factor *in vitro* and *in vivo.* Deletion of the hypoxia response element of the promoter region of VEGF in mice causes a reduction in neural VEGF expression, and results in adult-onset motor neurone degeneration that resembles amyotrophic lateral sclerosis (ALS). Understanding the molecular pathways to neurodegeneration in the VEGF$^{\delta/\delta}$ mouse model of ALS may improve understanding of the mechanisms of motor neurone death in the human disease.

Objectives: The mechanism of neurodegeneration in VEGF$^{\delta/\delta}$ mice is unknown. Chronic hypoxia has been proposed, as blood flow to neural structures is impaired. In addition to vascular development, however, VEGF plays a central role in the development of the nervous system, and may be required for survival of adult neurones. Disruption of these functions may cause neuronal degeneration in VEGF$^{\delta/\delta}$ mice.This study aimed to determine the pathways to neurodegeneration in the VEGF$^{\delta/\delta}$ mouse using transcriptional profiling of spinal motor neurones.

Methods: Spinal motor neurones were isolated by laser capture microdissection from 3 pairs of transgenic mice and wild-type littermates at 3 time points of disease. Extracted RNA was amplified, and gene expression profiles determined by microarray analysis, on the Affymetrix platform. Functionally important changes in gene expression were determined by analysis of gene ontology enrichment of significantly differentially expressed genes. A proportion of significant gene changes were confirmed by quantitative rtPCR.

Results: 324 genes were significantly differentially expressed in motor neurones of pre-symptomatic VEGF$^{\delta/\delta}$ mice, 384 genes at disease onset, and 689 genes at late stage disease. In early disease, there was transcriptional up regulation, with a switch to major transcriptional down regulation at late stage disease, associated with down regulation of genes involved in RNA processing. At symptom onset, the reduction in neural expression of VEGF observed in VEGF$^{\delta/\delta}$ mice was accompanied by down regulation of genes involved in the maintenance of neuronal processes and synapses: genes encoding cholesterol synthesis enzymes, synaptic proteins, cell adhesion molecules and proteins involved in neurite outgrowth and axonogenesis.

Discussion and Conclusions: The mechanisms involved in maintaining neuronal integrity once circuits are established, in the face of the demands of synaptic plasticity, is little understood despite their potential relevance to neurodegeneration. We have presented evidence that VEGF is required in the adult mouse for the maintenance of distal neuronal processes and synapses, such that a reduction in VEGF expression in VEGF$^{\delta/\delta}$ mice leads to axonal retraction and cell death.

C90 A HUMAN P190RHOGEF HOMOLOGUE, RGNEF, INTERACTS WITH HUMAN LOW MOLECULAR WEIGHT NEUROFILAMENT (NFL) MRNA

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Keywords: RNA stability, neurofilament, aggregates

Background: p190RhoGEF is an important modulator of low molecular weight neurofilament (NFL) mRNA stability in the mouse, with alterations in expression of p190RhoGEF leading to neurofilament (NF) aggregate formation. A C-terminal region of p190RhoGEF interacts with a small region of NFL mRNA extending from the 3' end of the coding region into the 3' untranslated region which results transcript stabilization. Alterations in the expression of NFL protein subunits appear to contribute to NF aggregate formation in murine models of ALS. To date, no p190RhoGEF homologue has been described in humans.

Objectives: To determine if a p190RhoGEF homologue is present in human brain and spinal cord, and if it can interact with NFL mRNA in a manner similar to that of murine p190RhoGEF.

Methods: Sequence data mining revealed the existence of up to four predicted human homologues of p190RhoGEF. Sequence analysis revealed that human RGNEF had similar protein structure and domain organization as murine p190RhoGEF, including the presence of a RNA binding domain in the C-terminal region of the protein. This domain was expressed as a GST fused protein, then gel shift assays were used to confirm interaction with human NFL mRNA. RT-PCR was used to detect RGNEF expression in human spinal cord and brain from both control and sporadic ALS (sALS) cases. GST-fused RGNEF protein was then supplemented to lysates from control (3 cases) and sALS (3 cases) spinal cords, immunoprecipitated, and the presence of NFL mRNA determined by IP-RT-PCR.

Results: RGNEF was expressed throughout brain and spinal cord tissues from control and sALS cases. The C-terminal region of RGNEF interacted with *in vitro* transcribed NFL mRNA as predicted. Interestingly, we detected strong interaction of RGNEF with NFL mRNA in sALS spinal cord lysates but not in control lysates. NFL mRNA expression was confirmed in all samples and found to be present in the supernatant fraction in control lysates, showing that the absence of interaction was not due to absence of NFL mRNA expression.

Discussion: These data indicate that RGNEF can interact with human NFL mRNA in a manner similar to that of seen with p190RhoGEF and murine NFL mRNA. RGNEF is also expressed in the brain and spinal cord from both control and

sALS patients. However, differences in interaction between control and sALS lysates with respect to NFL mRNA binding to RGNEF were seen.

Conclusions: While p190RhoGEF can directly contribute to the etiology of NF aggregates in mice, it also appears that the human homologue, RGNEF, is an important human NFL mRNA stability determinant. Differential NFL mRNA binding between control and sALS lysates suggests that RGNEF may play a similar role to that of murine p190RhoGEF and NF aggregate formation.

C91 CMT2E-CAUSING MUTATION OF NFL INTERFERES WITH MITOCHONDRIAL FUSION AND DISRUPTS A NORMAL FUNCTION OF NFL IN DETERMINING MITOCHONDRIAL MORPHOLOGY

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Keywords: sensorimotor neuropathies, neurofilament, mitochondrial fusion-fission

Background: Neurofilaments (NFs) are implicated in the pathogenesis of motor neuron disorders and in the preferential vulnerability of motor neurons to initiating factors. Abnormal accumulation of NFs or aggregation of NF proteins occurs in ALS and in the sensorimotor neuropathy, Charcot - Marie -Tooth disease, due to mutations in the NEFL gene (CMT2E). In CMT2E, mutations in the low molecular weight NF subunit (NFL) induce aggregation of NFs. However, we previously reported that shortening of mitochondria is observed prior to NF abnormalities in cultured motor neurons expressing NFL mutants. This mitochondrial rounding is reminiscent of another form of CMT2 caused by mutations in the gene encoding the pro-fusion protein, mitofusin 2 (MFN2). MFN2 mutants induce shortening and clustering of mitochondria, suggesting a common pathway between these forms of CMT2.

Objectives: To determine the normal role of NF proteins in the control of mitochondrial dynamics and how CMT2E-causing NFL mutants cause mitochondrial rounding.

Methods: To test the effect of individual NF proteins on mitochondrial morphology and distribution in comparison to MFN2 proteins, NFL, NFH, NFLQ333P (a CMT2E-causing NFL mutant), MNF2 or MFN2R94Q (a CMT2A-causing mutant that inhibits mitochondrial fusion) was co-expressed with mitochondrially targeted dsRed in SW13- cells (an adrenocarcinoma devoid of intermediate filaments). Mitochondrial length and clustering were measured by confocal microscopy. To quantify effects of NF and MFN2 proteins on mitochondrial fusion, each was co-expressed with a mitochondrial-targeted, photoactivatable eGFP in motor neurons of dissociated spinal cord cultures. A small area was photoactivated and fusion was measured as decay in the intensity of fluorescence of the spot by time-lapse confocal live imaging.

Results: SW13- cells expressing NFLwt had longer and more reticulated mitochondria, but not when fusion was prevented by co-expressing MFN2R94Q. Unlike NFLwt, NFLQ333P caused rounding and shortening of mitochondria similar to the MFN2 mutant. NFH had no effect on mitochondrial length, but rather promoted clustering of mitochondria. In motor neurons, mitochondrial fission/fusion was unaffected by over-expression of NFLwt, but was profoundly inhibited by expression of NFLQ333P, similar to MFN2R94Q.

Discussion and Conclusion: The data reveal involvement of NF proteins in mitochondrial dynamics, NFH influencing mitochondrial distribution and NFL influencing mitochondrial morphology, possibly by affecting the balance of fission/fusion. The CMT-causing mutant, NFLQ333P, induces rounding of mitochondria prior to NF disruption by impairing fusion/fission, an effect similar to CMT2A-causing mutations in the mitochondrial protein MFN2 and linking CMT2A and CMT2E to a common cellular dysfunction.

C92 THE LEGS AT ODD ANGLES MUTATION (LOA) RESCUES MITOCHONDRIAL FUNCTION IN SOD1G93A MICE

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Keywords: SOD1, Loa, mitochondria

Background: We and others have previously demonstrated that the F580Y point mutation in cytoplasmic dynein heavy chain (DYNC1H1) results in the loss of sensory and motor neurons in the *Loa* mouse. In addition, we have shown that the *Loa* mutation delays disease onset and motor neuron loss in double mutant (*Loa*/SOD1^{G93A}) transgenic mice, leading to a significant increase in life span in these mice.

Objectives: To determine the underlying molecular mechanisms of the improved mitochondrial function by the *Loa* mutation and its link with the amelioration of the disease phenotype in *Loa*/SOD1^{G93A} double mutants.

Methods: We used gradient sedimentation techniques to isolate enriched mitochondrial fractions from SOD1^{G93A} and double mutant *Loa*/SOD1^{G93A} mouse brain and spinal cord homogentes. This was followed by a cohort of biochemical assays including polarography and spectrophotometry, blue native gel and 2D-gel electrophoresis to compare the integrity, composition, and functions of the mitochondrial respiratory chain complexes and their potential association with mutant SOD1 in the two mouse strains at the late stage of the disease.

Results: Our data from spectrophotometric and polarographic assays indicate that while succinate dehydrogenase (complex II) activity is normal, the activity of cytochrome c oxidase (complex IV) is compromised in mitochondria isolated from SOD1^{G93A}. Importantly, these assays also demonstrate that complex IV activity is partially restored in *Loa*/SOD1^{G93A} mice. Moreover, the blue-native gel electrophoresis assays that we used to separate the mitochondrial respiratory chain complexes suggest strong association of mutant SOD1 with ubiquinone:cytochrome *c* oxidoreductase (complex III). Of particular importance was the finding that the association of ATP synthase (complex V) with mutant SOD1 is significantly reduced in the mitochondria isolated from *Loa*/SOD1^{G93A} brain homogenates.

Discussion and Conclusions: The amelioration of the disease phenotype in SOD1^{G93A} by defective dynein has highlighted the dynein mediated retrograde axonal transport as a potential target for therapy. The significant reduction in the association of mutant SOD1 with the mitochondrial fractions indicates a role for the *Loa* mutation in protecting

the mitochondria from $SOD1^{G93A}$ toxicity. Importantly, the impaired cytochrome *c* oxidase function in the $SOD1^{G93A}$ mitochondria and the partial rescue of this defect by the *Loa* mutation suggest that the amelioration of the disease phenotype by mutant dynein is at the mitochondrial level. Moreover, the reduced association of mutant SOD1 with complex V in the mitochondria from *Loa*/$SOD1^{G93A}$ mice indicates that the function of this complex could also be compromised in the $SOD1^{G93A}$ and that the *Loa* mutation, by depleting mutant SOD1 from these complexes, could restore their functions. We will present our findings on these and other components of the respiratory chain in Loa/$SOD1^{G93A}$ versus $SOD1^{G93A}$ mitochondria.

SESSION 10B RESPIRATORY MANAGEMENT

C93 RESPIRATORY EXERCISES IN AMYOTROPHIC LATERAL SCLEROSIS (REALS)

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Keywords: respiratory exercises, delayed-start design, neuroprotection

Background: Respiratory insufficiency is the main cause of death in ALS. Non-invasive ventilation and gastrostomy increase survival and quality of life in ALS. Without newer effective pharmacological interventions, it is fundamental to develop programs to improve respiratory function.

Objectives: The authors aimed to test a respiratory muscle-training program in ALS.

Methods: Inclusion criteria: age 18–75 years, disease duration <18mo, ALS-FRS 25–38, with definite or probable disease, written informed consent. Exclusion criteria: bicipital and flexor digiti muscular strength <4 MRC, weakness of lip sealing, forced vital capacity (FVC) <70%; maximal inspiratory (MIP) and/ or expiratory (MEP) pressures <50%, patients on NIV, gastrostomy, other concomitant diseases such as diabetes and pulmonary diseases, sternocleidomastoid (SCMAmpl) and diaphragmatic (PhrenAmpl) motor amplitudes <1mV or <0.3mV respectively. Patients performed an 8-month respiratory muscle strengthening programme using the "Threshold IMT®". They were randomized in 2 groups: the efficient load group (G1) and non-efficient load group (G2). However, patients in G2 also performed the exercise with efficient load in the last four months (delayed start study design). Efficient load was individually calculated as 30–40% from MIP and in the first 4 months patients in G2 worked-out with the lowest possible load. Patients were evaluated 3 times, at entry and every 4 months, with ALS-FRS, FVC, MIP and MVV (maximal voluntary ventilation), sniff nasal inspiratory pressure (SNIP), PhrenAmpl and SCMAmpl, VAS for fatigue and dyspnoea, subjective respiratory control feeling, Fatigue Severity Scale (FSS), Epworth's scale, Functional Independence Measure (FIM), Euro-QoL 5D and Hamilton's scale.

Results: Nineteen patients (13 men, aged 57.7±8.8yrs, 3 bulbar-onset form, 2 definite disease, mean disease duration of 13.2±7.7 months) were included, 4 dropped out due to rapid disease progression. There were no demographic differences between groups at entry. We observed a higher ALS-FRS and MVV decrease in G2 in the first four months. No other differences were found. All patients in both groups described a better voluntary control over respiratory dynamics.

Discussion and Conclusions: Exercise is controversial in ALS. Nonetheless, it seems that aerobic exercise at moderate load is related to longer survival and better quality of life. To our knowledge, there has been no specific respiratory exercise programme tested in ALS. In our study, patients in both groups referred a subjective improvement in the voluntary control of the ventilation, as well as a trend for a better outcome in G1 for ALS-FRS and MVV. However, these preliminary results show a trend towards a better outcome for patients included in G1. Although the completion of this investigation is essential before more definite conclusions, our findings suggest that these studies on respiratory exercise are relevant in ALS.

C94 HOME INITIATION OF NON-INVASIVE VENTILATION FOR MOTOR NEURONE DISEASE

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Keywords: non-invasive ventilation, district general hospital, home initiation

Background: The local MND team has been in existence since 1995. Based in a local District General Hospital (DGH), the team provides patient-centred, key worker-led multi-disciplinary team (MDT) care. As the team has developed so has the non-invasive ventilation (NIV) service. The service is unusual in that ventilation is usually initiated in the patients' homes. The team believes that home initiation of NIV by a DGH based team is at least as effective as initiation in a specialist secondary healthcare setting, and is popular with patients.

Objectives: To compare outcomes against published data from the Newcastle NIV study (1).

Methods: Retrospective caseload review of 42 patients who had died between 1996 and 31 March 2009 and who had had a trial of NIV was conducted. The case notes were analysed to include all patients who had NIV irrespective of type of presentation. The outcome was mean length of survival from initiation of ventilation to death. Data are also presented for survival of all patients.

Results: Bourke reported a survival of 219 days from initiation of ventilation. Our mean length of survival from initiation of NIV was 348 days. Our mean length of survival for all patients from diagnosis was 580 days. Over 90% of patients with MND within the service have NIV initiated at home. This avoids hospital admission for initiation of NIV. Our data indicate that it is safe and effective as mean length of survival is comparable to published data and patients prefer to have NIV initiated at home rather than in hospital. The level of patient satisfaction with the service is also very high.

Discussion: We have shown that home initiated NIV in MND is safe and effective over several years. The key factor in that success is the ability to monitor symptoms and detect the early onset of ventilatory failure in an MDT setting, using equipment such as transcutaneous monitoring of CO_2. The MND MDT is trained to recognize the early symptoms of respiratory failure. Early detection of symptoms is followed up by a team of specialist nursing staff with expertise in respiratory management. The team is based in a District General Hospital but outreach to the primary care setting. The respiratory team monitors patients regularly to optimize ventilatory settings and encourage early use of adjunctive therapies. This may include mechanical cough assistance and early antibiotic therapy.

Conclusion: Home initiation of NIV is safe and effective in MND.

Reference:

1. Bourke SC, *et al.* Lancet Neurology 2006, 5:140–147

C95 USUAL INDICATORS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) DISEASE SEVERITY DO NOT PREDICT OVERNIGHT EFFICACY OF SUBJECTIVELY PRESCRIBED NONINVASIVE POSITIVE PRESSURE VENTILATION

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Keywords: non-invasive ventilation, forced vital capacity

Background: Current guidelines for ALS recommend nocturnal non-invasive positive pressure ventilation (nNIV) for forced vital capacity (FVC) <50% predicted, to prolong survival, sustain respiratory muscle strength and improve sleep quality. nNIV is typically non-objectively prescribed and adjusted. The nocturnal physiologic efficacy of subjective nNIV use in ALS patients has not previously been assessed. Given the challenge of administering nNIV to such patients, we hypothesized that ALS patients commonly have failure of nocturnal oxygenation and ventilation with such nNIV, even when reporting adherence and subjective efficacy.

Objectives: To determine the efficacy of subjectively prescribed nNIV in ALS patients reporting adherence and benefit (improved dyspnea and/or sleep quality) with this treatment and ascertain if common measures of disease severity predict nNIV failure.

Methods: Twenty consecutive ALS patients reporting successful use of nNIV (>4 h/night, >4 nights/wk and subjective benefit) were prospectively recruited from the Eleanor and Lou Gehrig ALS/MDA Center, and underwent home nocturnal polysomnography (PSG) using their current nNIV regimen. PSG included airflow, NIV pressure, thoracoabdominal effort, ECG and pulse oximetry (SpO_2). nNIV failure was defined as O_2 desaturation index >4% (ODI4%) ≥5/h recording time; and/or ineffective ventilation time (patient-ventilator asynchrony, central apnea, delivered inspiratory pressure (IPAP) >2cm H_20 below set IPAP) >5% recording time. Data were analyzed with unpaired t-tests and Fisher's exact test.

Results: Twenty patients were studied (6F/14M, mean age 57±10.8, FVC 42.1% predicted±16.4, DFS 0.62±0.48). 9 of 20 patients (45%) demonstrated nNIV failure, with mean± SD nadir SpO_2 =80±6%, ODI 4 =6±4/hr, SpO_2 <90% = 6±6% recording time, ineffective ventilation time =18±16% recording time. nNIV failure and success groups were similar for age (failures 60.6±11.6 years vs 54.4±9.7 years, p =0.2), FVC at the time of PSG (failures 48±18% predicted vs 38± 14%, p =0.2), ALSFRS-R score at the time of PSG (failures 25±7 vs 22±9, p =0.5), rate of functional decline (DFS, failures 0.65±0.43 vs 0.60±0.54, p =0.8), bulbar onset (failures 56% vs 64%, p =0.9), presence of PEG (failures 33% vs 64%, p =0.9), level of set IPAP (failures 13±4cm H_2O vs 12±4 cmH_2O, p =0.6), and NIV duration prior to PSG (failures 9 ±5 months vs. 7±5 months, p =0.4). 5 subjects who did not meet minimal criteria for nNIV failure evidenced nadir O_2 saturation of <85% or ventilator double triggering >10 events/hr.

Discussion and Conclusions: These data suggest that current nNIV practice is likely not meeting the goals of improving sleep disordered breathing (SDB) or sustaining respiratory muscle strength for ~50% of patients prescribed such therapy. The prevalence of nNIV failure among all patients subjectively prescribed nNIV is likely higher than that seen in this data. Neither degree of disease progression nor impairment appears to predict nNIV success in this setting.

C96 MULTICENTER STUDY RESULTS OF MOTOR POINT STIMULATION FOR CONDITIONING THE DIAPHRAGM OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS/ MOTOR NEURON DISEASE: PRELIMINARY TREND TOWARD SLOWED RESPIRATORY DECLINE AND IMPROVED SURVIVAL

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Keywords: diaphragm pacing, respiration, surgery

Background: The diaphragm pacing system (DPS) is a standardized minimally invasive laparoscopic technique intended to maintain and provide natural diaphragm ventilation. Respiratory insufficiency through diaphragm dysfunction is the major cause of mortality in ALS/MND and presently available therapies are inadequate to address this problem.

Objectives: Assess the safety and efficacy of DPS for diaphragm conditioning in ALS/MND.

Methods: Prospective, nonrandomized, controlled, multicenter, interventional trial with a lead-in design and 9 month post -implantation treatment period. Prospective efficacy and safety measures were obtained including the rate of decline of pulmonary function, quality of life measurements, adverse event rates and survival or full time tracheostomy with mechanical ventilation.

Results: 145 subjects enrolled with 106 implantations from March 2005 to January 2009 (most common reason for not implanting was a drop of FVC below 45% of inclusion criteria). Subject demographics from the first 88 implanted patients are: mean age 54.9±10.3, 71.6% male, 27.9% bulbar onset, ALSRS-r total score 27.8±7.2, riluzole use of 74%, symptom onset to implantation 41.5 months ±27.1, FVC (% predicted) 61.1±11.8, average pCO_2 39mm Hg (max =60), SF 36 average physical of 36, SF 36 average emotional of 53, and non-invasive ventilation use of 82% throughout the study. With a cumulative 1,346 months of device usage (average 1.1 years/patient), safety analysis showed no serious unanticipated adverse device effects with 3 (2.8%) serious adverse events

related to surgical procedure. There were 17 reported respiratory events during the study period, and only one patient stopped pacing. Interim analysis shows a significant ($p < 0.001$) improvement (7%) in ratio of the ALSFRS-R respiratory subscore to the total ALSFRS-r score from implant to treatment. In the subset of subjects with at least 6 months of treatment data and a declining FVC during the lead-in period ($n = 45$), there was a significant reduction in FVC rate of decline ($p = 0.01$), with a paired (treatment to lead-in for each patient) FVC improvement of 1.1% change from 2.7% decline during lead-in to 1.6% decline post implantation. The 30 day survival was 100% (106/106), the 6 month survival was 92% (90/98) and the 12 month survival was 78% (56/72). Using Kaplan-Meir analysis the mean survival is 25.7 months post implant. When analyzing combined DPS and gastrostomy placement subset the 6 month survival was 89% (24/27) and the 12 month survival was 74% (17/23). Respiratory events accounted for 41% of the end events.

Conclusion: The DPS system can be safely implanted and utilized in ALS patients. DPS seems to have positive effects in maintaining respiratory function and decreasing respiratory decline in those declining prior to implant. Overall survival and survival with a gastrostomy seems higher than historical comparisons.

C97 DIAPHRAGM PACING IN ALS: PRELIMINARY RESULTS SUGGEST SIGNIFICANT SLEEP IMPROVEMENT

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Keywords: respiratory insufficiency, diaphragm, sleep

Background: During amyotrophic lateral sclerosis (ALS), respiratory insufficiency and diaphragmatic involvement cause major sleep disturbances. Their correction by non-invasive ventilation (NIV) largely contributes to the improvement in quality of life associated to this therapeutic. It has been suggested that diaphragm pacing (DP) using a laparoscopically implanted device (NeurRx, Synapse, Oberlin, OH, USA) could slow down decline in lung function. To test this hypothesis, a prospective, non randomized, multicenter interventional trial has been conducted among 106 patients at 8 centers. In the subset of patients implanted in Paris, we further tested the hypothesis that DP could maintain diaphragm strength and improve sleep.

Patients: 11 patients from the Paris cohort ($n = 18$) had completed 4 months of DP at the time of the present submission; 6 men, 5 women; median age 68 years, 95%CI 63–74; median ALSRS-r total score 34 (29, 40); riluzole 100%; NIV $n = 5$; bulbar onset $n = 1$; median interval between diagnostic and implantation 30 (19,42) months.

Methods: In addition to the evaluation of the rate of decline of lung function, quality of life, safety and survival, the Paris patients had measurements of twitch esophageal and transdiaphragmatic pressures (Pes,tw and Pdi,tw) in response to bilateral anterior magnetic stimulation (BAMPS) and sleep assessments including polysomnographic recordings (PSG) immediately before the implantation and after 4 months of diaphragm stimulation.

Results: During the conditioning period, forced vital capacity (FVC) significantly declined. There was no change in its rate of decline as compared to the lead-in period, although none of the patients had to start NIV. Pdi,tw and Pes,tw were markedly altered initially (median 6.7 (4.6, 15.0) cm H_2O and 2.3 (1.9, 7.0), respectively). In contrast to spirometric variables, they remained unchanged after 4 months. Median sleep efficiency increased from 67 (51, 71)% to 74 (70, 85)% ($p = 0.02$) with a significant reduction in sleep fragmentation (median arousal index from 28 (14, 30) to 13 (10, 21) events/h, ($p = 0.01$). The amount of non REM sleep stage N3, the amount of REM sleep, and sleep latency were unchanged, arguing against a first night effect. In coherence with these results, the patients reported lower Epworth scores after the 4 months pacing period (from 6 (3, 12) to 5 (3, 6), $p = 0.05$).

Conclusion: The quality of sleep significantly improved after 4 months of diaphragm pacing in a subset of the Paris cohort of the international DP trial in ALS. This was not associated with a significant reduction in the rate of decline of FVC, but the strength of the diaphragm did not decline during the conditioning period. The mechanisms of the improvement in sleep remain to be elucidated.

C98 SURVIVAL IN ALS PATIENTS AFTER TRACHEOSTOMY

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Keywords: tracheostomy, survival, prognosis

Background: The most common cause of death in patients with Amyotrophic Lateral Sclerosis (ALS) is respiratory failure due to progressive impairment of the respiratory muscles. The median survival is 36 months in ALS patients who do not receive invasive mechanical ventilation. Little information is reported in the literature about the survival of ALS patients who undergo tracheostomy.

Objectives: The aim of our study is to analyze the survival of ALS patients who receive pressure positive ventilation by tracheostomy.

Methods: 95 out of 646 ALS patients followed in our Center decided to receive mechanical ventilation by tracheostomy. Long-term follow-up of 85/95 patients was available and was analyzed in the present study. Survival was calculated using Kaplan-Meyer analysis.

Results: There were 48 males and 37 females. The age of onset ranged from 12–82 years (media 54.81, median 57 years); in 18 patients (21.2%) the disease began before 40 years, in 67 patients (78.8%) after 40. The site of onset was bulbar in 21 patients (24.7%) and spinal in the remaining 64 patients (75.3%). 81 patients had a sporadic ALS, while in 4 patients a family history was present. Dividing sporadic ALS group by clinical phenotype, we observed 62 patients (76.5%) with classic ALS, 18 patients (22.3%) with predominant upper motor neuron phenotype (p-UMN) and 1 (1.2%) with Flail Arm variant. The time from onset of disease to tracheostomy ranged from 6 to 134 months (mean 35.71, median 28 months). Before tracheostomy 24 patients used a NIV, for a mean duration of treatment of 8.84 months (range

1–21 months); only 2 patients used NIV 24 hours/24 before receiving tracheostomy, for 5 and 10 months, respectively. The median survival after tracheostomy was 40 months (95% CI 25.93–54.06 months). Age of onset before 57 years and the time from onset to tracheostomy longer than 28 months were significantly associated with longer survival. After tracheostomy, 10 patients (12%) had a survival longer than 5 years, while only one patient was alive after 10 years (1.2%). 4 patients (4.7%) developed a totally locked-in state after a mean period of 27 months (range 15– 48 months) after tracheostomy and 45 months (range 27–69 months) from onset of disease.

Discussion and Conclusions: In our series, the median survival of ALS patients receiving tracheostomy is 40 months. Age of onset of the disease and the period of time from onset to tracheostomy are significant prognostic factors. The knowledge of the evolution of ALS after tracheostomy may be helpful in the hard task of end-of-life decisions.

C99 A POPULATION-BASED STUDY OF TRACHEOSTOMY IN ALS

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Keywords: tracheostomy, incidence, outcome

Objectives: Respiratory failure is frequent in amyotrophic lateral sclerosis (ALS). Its management is based on mechanical ventilation with non-invasive positive pressure (NIPPV) or tracheostomy when NIPPV is no longer effective. However, information about the outcome of tracheostomy in ALS is scarce.

Methods: We evaluated the clinical characteristics and outcome of tracheostomy in ALS using data from the Piemonte and Valle d'Aosta Register for ALS (PARALS), a prospective epidemiological register collecting all ALS incident cases in two Italian regions.

Results: Among the 1260 patients incident in the period 1995–2004, 134 (10.6%) underwent tracheostomy. Young male patients were more likely to be tracheostomized. Site of onset (bulbar vs. spinal) and period of diagnosis (1995–1999 vs. 2000–2004) did not influence the likelihood of being tracheostomized. The mean duration of hospital stay was 52.0 days (SD 60.5). Overall, 27 patients died while still in hospital (20.1%); in-hospital mortality was lower in subjects followed at ALS multidisciplinary centers (13.1% vs. 28.8%; $p = 0.02$). Sixty-five patients (48.5%) were discharged to home, while 42 (31.3%) were admitted to long term care facilities. The median survival time after tracheostomy was 253 days. In the Cox multivariable model the factors independently related to a longer survival were enteral nutrition, age, marital status, and ALS centre follow up.

Conclusions: In an Italian epidemiological setting ALS survival after tracheostomy was less than one year. Sociocultural factors may influence the probability to be tracheostomized, even in a highly socialized health system such as the Italian one.

SESSION 11 JOINT CLOSING SESSION

C100 CLINICAL BIOMARKER USE IN THE CLINICAL INVESTIGATION OF NEURODEGENERATIVE DISEASE

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Keywords: biomarker, pharmacodynamic, biological activity

The ultimate goal of clinical science is to identify novel therapeutics to relieve human suffering. Therapies for neurodegenerative diseases, including ALS, aim to arrest or slow the progression of disease and the worsening of disability.

Rational drug development identifies biological targets that may be important to a disease's pathophysiological process and then creates interventions that impact these targets. The key challenge to drug development is the translation of these discoveries from the laboratory bench into the human patient. It is in this endeavour that clinical biomarkers can be used. In considering clinical biomarkers it is important to distinguish between pharmacodynamic markers that measure the biological effect of a therapeutic intervention and other biomarkers that reflect the pathophysiological processes of the targeted clinical disease. A pharmacodynamic biomarker allows the investigator to ascertain if the study drug is interacting with and affecting its desired target and helps to identify the dosage range and exposures required to affect this target. A pharmacodynamic marker will not necessarily predict a therapeutically meaningful effect in the studied disease and population, but it will allow the investigator to confirm that the biological hypothesis has been tested in clinical trials leading to a definitive "positive" or "negative" outcome. Such a clear binary outcome is eminently more desirable than a "failed" study where the clinical outcome in the disease population is negative but it is not known if the targeted biology was altered by the study drug. Examples of pharmacodynamic markers include assays of dystrophin in studies of therapeutic ribosomal read-through of premature termination codons in Duchenne muscular dystrophy and Ab clearance from the brain in Alzheimer's disease. Biomarkers that reflect the pathophysiological process of the target neurodegenerative disease are used to identify the optimal test population for a new therapeutic, to monitor disease progression and to measure slowing of disease progression. Examples of biomarkers of disease pathophysiology include MRI brain lesion number and volume in multiple sclerosis (MS), Pittsburgh B PET scanning to confirm the presence of Ab plaque in the brain and viral load in HIV/AIDs. In some instances, changes in these biomarkers (MS MRI, HIV viral load) are also highly predictive of a clinical effect.

Identifying biomarkers is a very high priority for the neurodeneration field, in general, and the ALS field, in particular. For timely delivery of such biomarkers, their development should occur in parallel with transition of a molecule from non-clinical to clinical development.

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THEME 1 THERAPEUTIC STRATEGIES

P1 THE COCHRANE COLLABORATION – A DECADE OF EVIDENCE-BASED REVIEWS IN ALS/MND

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Keywords: cochrane collaboration, evidence-based medicine, ALS management

Background: The Cochrane Collaboration is a global voluntary scientific network dedicated to making up-to-date, accurate information about the effects of therapeutics readily available worldwide.

Objectives: To review the progress of the Cochrane Neuromuscular Research Group (NMRG) that evaluates various therapies for ALS/MND.

Methods: Each Cochrane systematic review utilizes the standard methodology of the Cochrane Collaboration including a software program for statistical analyses of the reported data from relevant randomized-controlled clinical trials. Interested reviewers meet annually at the International ALS/MND meeting to share progress and plans. The editorial office in London, UK, provides support for literature searches and editorial assistance.

Results: To date there have been 11 systematic reviews and 10 protocols published in the Cochrane library (issue 1, 2009). The systematic review of riluzole has been recently revised to include recently published data from large registries. The review of insulin-like growth factor-1 examined the evidence in two published trials, pointing out methodological concerns, soon to include the third recently completed IGF-1 trial. Earlier reviews of antioxidant treatment and ciliary neurotrophic factor were published.
Within the past year, three systematic reviews appeared: clinical trials in familial ALS, exercise in ALS/MND and treatment for pain in the terminal phase of ALS/MND. In the familial ALS review the authors found no difference between familial and sporadic patients in clinical trial responses, but point to the need for inclusion of familial cases, for trials to document whether or not ALS is familial and for sharing of data once trials are completed. The review of exercise, based on two small studies, found no harmful effects of exercise and some modest benefits, but larger studies are needed. The review of pain treatment pointed to the lack of controlled studies in ALS and the need to move forward with trials in this important area.
Two reviews on management issues were recently published, one for enteral feeding and the other for spasticity. In the review of spasticity, patients who were performing twice daily exercise had significantly less spasticity after three months than those who were not.
Protocols that have been published with reviews in progress include: breaking the news; treating sialorrhea; mechanical ventilation; multidisciplinary clinics and clinical trials of minocycline, gabapentin and creatine.

Discussion: Since the first systematic review of riluzole in ALS/MND in 1999, there has been a steady increase in the number of reviews. A substantial number of new reviews have appeared or are close to publication. The quality of clinical trials has improved, but reviews of management highlight the paucity of strong evidence in ALS therapeutics and point the way toward future research in this field.

Conclusion: More high quality clinical studies are needed to guide management in ALS.

P2 ONLINE SURVEYS OF PALS REVEAL THEIR OPINIONS ON ALS CLINICS AND RESEARCH STUDIES

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Keywords: clinics, trials, enrolment

Background: Less than 50% of North American patients with ALS (PALS) get care in a specialized ALS clinic. Only 10–25% of PALS in these clinics participate in research studies. Meta-analyses suggest that trial design factors do not account for low and variable enrolment in ALS research studies; rather, "doctor factors" and "patient factors" may be responsible.

Purpose: To gain a better understanding of the opinions of PALS toward specialized ALS clinics and the research offered there and ultimately to develop educational interventions that improve attendance in ALS clinics and enrolment in ALS research studies. Similar surveys have been used in cancer patients and led to interventions that improved enrolment in cancer studies.

Methods: An online survey directed toward PALS is underway, hosted by the website Survey Monkey. This consists of questions related to clinics, research studies and demographics and has been advertised on a large number of individual North American ALS Clinic websites (for example www.dukealsclinic.com) and on MDA and ALSA websites. Data from the survey will be analyzed using simple descriptive statistics.

Results: The survey has been open and advertised since 29 December 2008. To date, 77 PALS have participated in this survey.
Participants thus far were white (100%) males (55%), mean age 57, with college or higher education (76%). A surprising percentage did not know that specialized ALS Clinics existed (15%). Those who did, usually learnt about them from their first neurologist (64%). Those who came to specialized ALS Clinics did so primarily for the multidisciplinary team (77%).

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The biggest complaints about the clinics were the amount of driving (50%) and time required to attend (30%). Information about research was obtained primarily from ALSA and MDA websites (59%) and support groups (48%). Only half the responders had ever been asked to be in a research study and of those asked 80% agreed to participate. Most common reasons for participating were beliefs that the study would help others (85%) and that it would help the participant themselves (73%). The majority of those who participated in a research study stated that they were never asked to participate again (74%). Most participants had not participated in online research studies, but said that the results of an online study would not affect their interest in a clinic based study.

Discussion and Conclusion: Even this preliminary data suggests that changes can be made to optimize enrolment in clinics (better advertising and geographic distribution, shorter clinic visit times) and enrolment in research studies (asking more often and creating a registry of past participants).

P3 UPDATE ON ALSUNTANGLED; A GLOBAL SCIENTIFIC EFFORT TO INVESTIGATE ALTERNATIVE AND OFF-LABEL TREATMENT OPTIONS FOR PATIENTS WITH ALS

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Keywords: ALSUntangled, internet, alternative therapies

Background: Patients with ALS (PALS) often consider alternative and off-label treatments (AOTs) they read about on the internet. These are high stake decisions, as AOTs are can be expensive and harmful; pursuit of an AOT is often undertaken in place of participation in a clinical trial (potentially delaying the search for better ALS therapeutics). Unfortunately, the evidence presented for the efficacy and safety of these typically ranges from scant to frankly inaccurate.
In April 2009, we initiated a system by which the community of ALS clinician scientists might work more closely and regularly together to systematically assess AOTs, ultimately helping PALS make more informed decisions about their care. This system, collectively referred to as ALSUntangled (ALSU) operates using newer internet tools. There are three parts to ALSU: learning about the AOTs that PALS are considering, a "war room" in which these AOTs are investigated by ALS Clinician Scientists and finally, public release of the results of the investigations. The first part utilizes Twitter; the second part utilizes an invitation-only site called NING and the third part utilizes free access publications via the journal Amyotrophic Lateral Sclerosis.

Objectives: To remind people of the availability of ALSUntangled and review early experiences with ALSUntangled.

Methods: Components of ALSUntangled were reviewed, including how PALS and ALS clinicians and scientists can access it. The number of "tweets" received, the amount of activity on the NING and the number of free access publications via the journal Amyotrophic Lateral Sclerosis will be reported.

Results: Thus far we have received 4 ALSUntangled tweets, all from members of the ALSRG with requests to evaluate Lyme Disease treatment, the X-Cell Center, the Hickey Wellness Center and Dr. Zannos Grekos' Stem Cell Clinic in Dominican Republic. The NING has attracted 20 members, with 4 forum topics being actively discussed (Lyme Disease treatment, the X-Cell Center, the Hickey Wellness Center and Dr. Zannos Grekos' Stem Cell Clinic in Dominican Republic). One free access publication from the program is scheduled for June 2009 (an introduction to ALSUntangled).

Discussion and Conclusions: ALSUntangled fills an important need: to facilitate organized scientific investigation of AOTs, which in turn can help PALS make more informed decisions about their care. Early experience is promising. In the ensuing months, we will begin to advertise the program and hope to see increased numbers of tweets and increased activity on the NING, which will result in more free access publications to help PALS and their providers.

P4 ENROLMENT IN THE STAR TRIAL

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Keywords: zenvia, enrolment, trials

Background: Surveys suggest that only 10–25% of PALS enrol in clinical trials; a meta-analysis revealed an average trial enrolment rate of only 2 subjects/site/month. These studies suggest tremendous variability in enrolment across sites (range 0–75%) and across trials (0.1–7.5 subjects/site/month). This variability is not explained by trial design factors, for example presence of placebo, or invasiveness of the intervention under study. "Doctor factors" and "patient factors" may be responsible.
We reviewed enrolment data from the STAR Trial. There were several differences between this trial and the previous trials reviewed in the above retrospective analyses. STAR was a trial of symptomatic (not disease-modifying) therapy and had more preliminary human data. STAR enrolled both PALS and patients with MS and utilized sites across North and Latin America. Finally, awareness initiatives for STAR were more extensive than previous trials, including the use of web-based advertising through Patients-Like-Me (PLM).

Methods: Enrolment data from the STAR trial were obtained from Avanir. Simple descriptive statistics were used to analyze these data and compare them to similar data from prior published ALS trials.

Results: The percentage of patients screened for this trial was not available, since the number of patients being followed at each clinic was not recorded. The percentage of screened patients that were enrolled was 79.7% (326 enrolled/409 screened). Overall the enrolment rate was 0.33 subjects/site/month (326 subjects/65 sites/15 months). Enrolment rate did not differ between North American sites (0.32 subjects/site/month; 224 subjects/47 sites/15 months) and Latin American sites (0.38 subjects/site/month; 102 subjects/18 sites/15 months). Enrolment rate did not differ between PALS and patients with MS, either. ALS enrolment rate was 0.24 subjects/site/month (197 subjects with ALS/55 sites enrolling either ALS or both/15 months) and MS enrolment rate was 0.34 subjects/site/month (129 subjects

with ALS/25 sites enrolling either MS or both/15 months).Variability in enrolment across sites was more impressive, ranging from 0 to 1.6 subjects/month.

Discussion and Conclusions: As seen in previous studies, the percentage of screened patients that enrolled in STAR was high; some "pre-screening" may have occurred to explain this (for example, not offering the study to patients who seem unlikely to qualify at first glance). Enrolment rate in the STAR trial was within the range seen in previously published trials of disease modifying therapies; also, the variability in enrolment across sites was much higher than the variability in enrolment across countries or across disease status. These findings support the previous hypothesis that trial design factors are not as important as "patient factors" and "doctor factors" in influencing enrolment. Surveys are underway to better understand patient and doctor factors, with the hope of developing strategies that enhance enrolment in future trials.

P5 A COMPARISON BETWEEN ALS PATIENTS PARTICIPATING IN CLINICAL TRIALS AND THOSE WHO DO NOT IN A LARGE MULTICENTER ALS CLINIC

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Keywords: clinical trials, recruitment strategies, disease progression

Background: Advances in basic science have led to an increase in the number of ALS clinical trials either currently being conducted or in planning stages. Within any clinical trial site, however, only a minority of patients are ultimately enrolled in trials. Understanding the differences between patients who are ultimately enrolled in trials and those who are not is critical for maximizing trial enrolment. In addition, any significant difference between these groups may have implications for how research findings may be generalized to the entire ALS patient population.

Objectives: To compare patient attributes between patients ultimately enrolled in clinical trials and those who are not, in a large multispecialty ALS clinic.

Methods: All patients seen at a large multispecialty ALS clinic were consecutively evaluated from June 2005 until May 2009. Baseline characteristics and measures of rate of progression (ALS-FRS-R) were obtained at clinic visits and differences were assessed between those patients who never participated in a clinical trial (Group 1) and those who did (Group 2).

Results: A total of 238 new patients were seen during the above time period. Of these, 72 (30%) participated in clinical trials. Average age at presentation was 60.7 (SD = 15.9) years for those not in trials and 56.7 (SD = 9) for those who did. Average ALSFRS-R at presentation for Group 1 was 34.2 (SD = 8.2) and 38.7 (SD = 5.1) for Group 2 and average vital capacity was 72.3% (SD = 26.4) for Group 1 and 84% (SD = 20.1) for Group 2. Rate of progression was 1.01 (SD = 0.96) ALSFRS-R units per month for Group 1 and 1.09 (SD = 0.75) for group 2, while change in vital capacity was 2.6% (SD = 3.9) per month for Group 1 and 2.7% (SD = 2.76) for Group 2.

Discussion and Conclusions: In the last 4 years, 30% of patients seen were ultimately enrolled in clinical trial. This is a markedly higher rate than previously reported and is likely due in part to the large number of trials enrolling at the center. Subjects who enrolled in trials were somewhat younger than those who did not, but rate of progression as measured by pulmonary function and ALSFRS-R was similar in the two groups. Of note, there was approximately 35% more variability in progression rate in those patients not in trials. One possible explanation for this is that patients either progressing faster or slower than average are less likely to be able to access trials.

P6 MINIMIZING ADVERSE EVENT REPORTING BIAS IN A HISTORICALLY-CONTROLLED SCREENING TRIAL

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Keywords: clinical trial, historical controls, bias

Background: A new Phase II screening trial paradigm using historical controls has been designed in order to more efficiently identify promising candidate therapies in ALS. The first such screening trial of lithium carbonate is underway, using the placebo group from the recent minocycline trial as the control group. Determining safety by comparing the lithium carbonate arm to a historical control group raises special challenges.

Objectives: To report on the empirical analysis of potential biases surrounding the comparison of safety data from non-concurrent clinical trials.

Methods: Since the lithium trial began, ongoing analyses of safety data have been performed. When designing the trial, measures were taken to ensure that safety reporting would parallel the previous trial for a fair comparison. These included using similar inclusion and exclusion criteria, creating safety forms that were similar to the original trial, tracking adverse events (AE) and serious adverse events (SAE) at each visit over the same timeframe and instructing centers on how to collect safety data. The demographics and markers of disease severity were evaluated in order to form a comparative picture of both patient populations and to compare AE, SAE and mortality rates. Differences in safety reporting procedures were separated between trials in order to determine what variables can and cannot be controlled.

Results: Several possible issues that could lead to inconsistencies in AE reporting were identified. There were slight differences in AE forms because different types of side effects were expected in each trial. As some of the study sites and coordinators differed between trials, AE interview styles may differ. There may have been slight differences in the frequency or manner that the coordinating center reminded sites to enquire about AEs. The lithium patient population know they are on drug versus minocycline where there was a possibility of placebo. These biases seem to have less of an effect on the more severe markers of safety such as SAEs, unexpected increases in rate of progression and death where comparison to the historical placebo data appears robust.

Conclusions: Several potential biases were identified; however, many can be corrected. These results highlight the importance of standardizing AE reporting methods in order to

ensure that safety comparisons to placebo are accurate. Known side effects of the agent must also be considered to obtain an accurate estimation of whether the side effects are those that are expected. Finally, safety data must be viewed within the context of the risk benefit analysis of the study as a whole.

P7 SELECTION TRIAL DESIGN FOR PROMISING THERAPEUTICS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: selection design, Phase 2, drug development

Background: The process of developing new drugs for ALS is particularly challenging because of the relative rarity of this disorder and the resulting limited number of patients eligible for pharmaceutical trials. At least 32 unique compounds have been tested in phase II/III clinical trials with largely negative results. Given the poor success rate, new initiatives are necessary to optimize early clinical trial design to optimize the limited pool of resources.

Objectives: To use a selection trial as a means of choosing a treatment that indicates superiority to other treatments. This is fundamentally different from a conventional phase II trial that uses a formal hypothesis testing approach. The hypothesis approach requires data to prove significant difference in efficacy between the two treatments before a selection can be made. In contrast, a selection trial is designed to choose a treatment that indicates superiority over other treatments. If one in ten ALS drugs are effective and each drug is tested against placebo at 80% power, an average of $1/(0.8*0.1) = 12.5$ clinical trials would be necessary to find an effective drug. A multi-drug selection design can considerably reduce this time.
Since selection is made between active treatments, the selection procedure provides no information as to whether the selected treatment is beneficial, harmful, or neither. Therefore, a secondary non-superiority test against placebo should be conducted.

Methods: In the proposed selection design phase II schema, efficacy of multiple active drugs shown to be efficacious in models (*in vitro* and *in vivo*) of ALS will be compared. Primary efficacy will be measured by analyzing the rate of decline in the revised ALS functional rating scale (ALSFRS-R) slope in the different treatment arms. For the three-arm selection trial, there will be 30 participants in each arm. With a total sample size of 90 participants, there would be an 83% chance that the best of the three treatments would be chosen if one of the three treatment groups has a 30% decrease in the rate of ALS-FRS (or equivalently, a 0.3 decrease in slope) when compared to the other two treatments after six months.

Results: Assuming a six-month (26 weeks) three arm selection trial is conducted, with 30 participants in each of the three treatment arms and ALSFRS-R measurements at 0, 1, 2, 4 and 6 months; the trial would take only 65% of the time as a conventional Phase II study (consisting of a placebo and one treatment arm) would take to find an effective treatment, with a comparable sample size of 228 patients in the conventional Phase II trial to achieve similar power.

Conclusion: Selection design can speed the search for effective drugs in situations where there are several new treatments to be tested.

P8 AN HISTORICAL DATABASE OF PLACEBO CONTROLS FOR PLANNING CLINICAL TRIALS IN ALS

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Keywords: database, trials, model

Background: Many large phase III clinical trials have been carried out since riluzole was approved for ALS and all have been negative. With many compounds to evaluate, more efficient trial designs, including some that will utilize historical controls, are needed to find drugs that are promising, prior to launching large, expensive phase III trials.

Objectives: To develop a database of historical placebo controls to compare with patients receiving treatment in phase II ALS trials. To examine the controls for systematic changes over time and differences among different treatment centers. To use this database to plan phase III trials and confirm results of computer simulations.

Methods: With IRB approval, we have begun to assemble a large database of placebo patients from completed ALS clinical trials. This database can be used to design clinical trials in ALS and is currently being used as a source of historical controls for a phase II trial of lithium. Patient characteristics at enrolment included in the database are: age, first symptom (bulbar or limb), time since first symptom, time since diagnosis, FVC and ALSFRS. Periodic follow-up data include: ALSFRS or ALSFRS-R, FVC, muscle strength, weight loss and vital status at last follow-up. Computer models for power calculations were based on hypothesized distributions of drug efficacies and multi-stage testing.

Results: The database currently includes de-identified data from 748 placebo patients enrolled in 7 randomized clinical trials of treatments for ALS. Results to date show: rates of decline as measured by ALSFRS and ALSFRS-R have not changed over the 10-year period 1997-2007. ALSFRS/ALSFRS-R slopes vary with symptom duration and initial values of ALSFRS/ALSFRS-R and FVC, but are independent of age, sex and site of onset. Survival time depends on patient age, initial FVC, initial ALSFRS/ALSFRS-R and symptom duration. There are large, statistically significant differences in survival but much smaller differences in ALSFRS slopes among the different trials. The differences in slopes can be explained by differences in initial patient values, but differences in survival are not so readily explained. Through computer modelling, the number of patients required to discover an effective drug for ALS can be reduced by a factor of 2 or more using historical, in place of concurrent, controls in phase II screening trials prior to definitive phase III trials.

Discussion and Conclusions: A well-defined database of patient data from clinical trials has already proven to be useful in planning clinical trials for ALS. We look forward to expanding the database, which we will make broadly available,

to include patients from additional clinical trials so that we can continue to examine the variability of commonly used measures such as ALSFRS-R over time and among different trial centers.

P9 CENTRAL VENOUS CATHETER STUDY DRUG SELF ADMINISTRATION IS SAFE IN ALS

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Keywords: Hickman, safety, care

Background: In the multicenter, adaptive design clinical research trial of ceftriaxone in ALS, study participants and caregivers were taught central venous catheter (CVC) care. Each site is comprised of an ALS nurse; physician's assistant; and infectious disease, interventional and ALS physicians. Teaching included standard protocol for clean and sterile technique, how to self administer study medication twice daily via CVC via mechanical timed pump. Study participants and caregivers were taught signs and symptoms of infection, possible complications related to CVC and to report findings to study staff immediately. A questionnaire was administered at the end of training to establish study participant's understanding of CVC care.

Objectives: To demonstrate that through proper education by licensed personnel, CVCs are a safe route of "self" drug administration in clinical research. To make neurology researchers aware of the feasibility of CVC usage in future research studies broadening the scope of potential investigational drugs.

Methods: Hickman CVC safety data were collected for 66 participants. Safety was assessed by adverse events deemed by the site investigators to be related or possibly related to the CVC.

Results: The 66 participants cumulatively had 24,826 days of intravenous treatment. There were 10 serious adverse events related or possibly related to CVC, an incidence of 0.4/1000 days. These included 8 reports of bacteremia incidence of 0.3/1000 days and one pulmonary embolism incidence of 0.04/1000 days. There were 8 exit site infections (non-serious); incidence of 0.3/1000 days. There were 11 line breaks or displacements; incidence of 0.44/1000 days.

Discussion: It is possible to safely administer study drug twice a day by teaching caregivers to perform the administration. The risk of bacteremia from a CVC is related to the length of time that the catheter remains in place. The safety of home administration of study drug by trained caregivers was excellent; catheter related infections occurred at a rate of 0.4 infection per 1000 days of catheter use. This is lower than infection rates of 2.9 to 11.3 per 1000 catheter days reported by Center for Disease Control in their guidelines for prevention of intravascular catheter-related infections. In a review of the literature, only one study had a lower rate of 0.34 per 1000 catheter days in a cohort 50,470 patients treated in the home setting. Additional education to minimize line breaks and tears is in place for the next phase of the study.

Conclusion: CVCs are safe if study subjects and/or caregivers are educated for CVC care and followed by licensed personnel. Future research should incorporate these findings when designing clinical research trials.

P10 SAFETY, DOSAGE, TOLERABILITY AND PHARMACOKINETICS OF TETRAHYDROCANNABINOL (THC) IN ALS-PATIENTS

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Keywords: cramps, tetrahydrocannabinol, pharmacokinetics

Background: About 10% of ALS patients use Cannabinoids for control of symptoms such as muscle cramps, spasticity and dyssomnia (1). Furthermore, Cannabinoids may also be an important disease modifying medication, as they are neuroprotective in mice (2).

Objectives: To evaluate the safety, dosage and side effects of 5mg and 10mg THC in ALS patients.

Methods: A subgroup of 7 of 24 ALS-patients (enrolled in a randomized double-blind placebo-controlled crossover study to evaluate the efficacy of THC for cramps) were evaluated. 5mg of THC (Dronabinol®) were orally ingested on the first and 10mg on the second day, respectively. THC and its metabolites were measured before and at 30, 60, 120, 240 and 480 minutes after ingestion by using gas chromatography-mass spectrometry (GC-MS) as previously published (3). At the same interval, pulse and blood pressure were measured. At 480 minutes, a standardized questionnaire concerning side effects was answered (VAS; 0 to 10 points).

Results: Mean THC-plasma levels rose from 0 to 0.7ng/mL. Mean pulse rose from 81 bpm to 90 bpm with 5mg and from 79 bpm to 90 bpm with 10mg THC, respectively. Only one patient experienced tachycardia of 105 bpm. Three patients had an exacerbation of their (already at baseline elevated) systolic and diastolic blood pressure. Concerning side effects, 5mg were well tolerated by all patients. At 10mg almost all patients had side effects. Sleepiness, euphoria and drowsiness were the most common complaints.

Discussion and Conclusions: Single doses of 5mg were well tolerated, but unlike in healthy controls, doses of 10mg were much less well tolerated. This suggests that affinity to Cannabinoid receptors (CB1 and CB2) is different in ALS patients. THC is known to have central sympathomimetic properties and at 10mg, but not 5mg, blood pressure has to be controlled carefully in patients with arterial hypertension. The potential use of THC in ALS patients will be discussed.

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P11 A DOUBLE BLIND, MULTI-NATIONAL, PLACEBO CONTROLLED TRIAL OF TALAMPANEL IN AMYOTROPHIC LATERAL SCLEROSIS: STUDY DESIGN AND SUBJECT BASELINE CHARACTERISTICS

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Keywords: clinical trial, glutamate, Talampanel

Background: Glutamate mediated excitoxicity plays an important pathogenic role in the progression of patients with ALS. Both glutamate receptor antagonists and drugs that up-regulate glial glutamate transport proteins prolong survival and improve function in the SOD1 transgenic mouse models. Talampanel, an orally available selective AMPA antagonist with CNS penetration, shows efficacy in multiple models of epilepsy and neuroprotection. A pilot study of Talampanel in ALS patients showed promising results in reduction rate of progression as measured by ALSFRS.

Objectives: To study the safety, tolerability and efficacy of orally administered Talampanel in subjects with ALS as compared to placebo, in a 12 month global study run concurrently in North America, Europe and Israel.

Methods: Subjects were recruited from 25 clinical trial sites in Belgium, France, Germany, Israel, Italy, Spain, Netherlands, Hungary, USA and Canada. Eligible subjects with definite, probable or laboratory probable ALS and a slow vital capacity of $>70\%$ of predicted were randomized into 3 groups: placebo, talampanel 25mg three times a day by mouth (PO TID), or talampanel 50mg PO TID. The primary outcome measure is the rate of change of ALSFRS-R as compared to placebo. Secondary outcome measures include survival, change in strength as measured by manual muscle testing, vital capacity and quality of life. Safety testing includes serial ECGs, clinical laboratory assessments, adverse events and routine medical monitoring. Last visit for the last patient is expected in April 2010.

Results: From 29 September 2008 to 31 March 2009, 559 subjects were enrolled (196 (35%) females; 363 (65%) males). The average number of subjects/site/month (enrolment rate) ranged between 2.28 to 7.77 across countries. Average age was 55.9 years (range: 18.9–80.8). Subjects experienced their first ALS symptom 18.7 ± 8.4 months prior to study entry and had a diagnosis of ALS for 8.1 ± 6.7 months prior to study entry. Mean Body Mass Index (BMI) at entry was 25.6 ± 3.4 for males and 25.1 ± 4.9 for females. Mean ALSFRS-R at entrance was 38.4 ± 5.1 (range; 20–47), mean strength across 22 muscle groups was $91.0 \pm 14.4\%$ and mean vital capacity at entrance was 93.1% (range; 68–164%). The mean number of patients per North America (NA) site was 13 (range: 5–25). The mean per Rest of the World (ROW) site was 29 (range: 11–52). There were no apparent clinically significant variations in subject characteristics between NA and ROW (e.g. time from first symptom to diagnosis, BMI, ALSFRS-R and gender).

Discussion and Conclusions: The rapid enrolment of this study demonstrates the feasibility of performing international clinical trials in ALS. The subject's entry characteristics across countries will be discussed and will be compared to other recent ALS clinical trials.

P12 TOLERANCE AND SAFETY OF CDP-CHOLINE (CERAXON) IN MND

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Keywords: therapy, CDP-coline, neuroprotection

Background: There is evidence that *in vitro* CDP-choline protects motor neurons against apoptotic changes of chronic glutamate exitotoxicity. In MND this effect could be synergistic to riluzole, a proven therapy in MND. CDP-choline clinical trials are ongoing in both ischemic and hemorrhagic stroke. Safety and possible efficacy trials had been promising in clinical stroke. Up to now there are no data concerning CDP-choline in MND.

Objectives: To prove the hypothesis that CDP-choline in patients with MND is safe and well-tolerated even in combination with riluzole. This is the assumption for a CDP-choline efficacy trial.

Methods: After giving their informed consent 10 consecutive patients with sporadic MND were included in this study. Three patients were primarily bulbar. Seven patients were continuously treated with 100 mg/day riluzole. Patients with the necessity of tube feeding were excluded because of missing data about bioavailability of tube fed CDP-choline. At baseline all patients received a complete clinical examination, a blood sample and a questionaire covering all potential side effects.
Patients received oral CDP-choline in a dosage of 1500mg/day corresponding to that for the treatment of stroke. All previous medication including riluzole remained unchanged. Any new medication in the observed time interval was avoided.
The second assessment as performed at baseline followed after 6 weeks of treatment with CDP-choline.
Both assessments were compared intra-individually. Negative progression of any of the assessed parameters was listed and interpreted. The potential worsening of the neurological findings was refered to MND, if they could be classified as MND specific.

Results: The clinical examination revealed no side effects of CDP-choline. There were no cutaneous or mucosal reactions. The blood sample showed no negative changes of blood count, liver or kidney function or signs of inflammation. The questionaire revealed no symptoms related to the CDP-choline treatment. There was no measurable interaction in the patients with combined CDP-cholone and riluzole therapy. One patient complained of a headache but showed progressive sleep related hypoxic periods. The headache disappeared after ventilatory assistance. One patient had a gastrointestinal infection in the observed time interval,

but tolerated CDP-choline well before and after the infection. All but the one patient with the headache summarized the treatment with CDP-choline as positive and wished to be included in the following efficacy pilot study.

Discussion and Conclusions: CDP-choline was safe and well-tolerated in a small number of patients with MND in a short time period of observation. This result is in accordance with the data from CDP-choline studies in acute stroke. Furthermore a dual glutamate exitotoxicity inhibition with CDP-choline and riluzole is well-tolerated in MND. As a consequence a CDP-choline efficacy pilot trial has started.

P13 THE POSITIVE EFFECTS OF MEMANTINE ON TAU ASSOCIATED NEURONAL DEGENERATION IN ALS: AN OPEN LABEL PILOT TRIAL

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Keywords: Rilutek, memantine, biomarkers

Background: There are several lines of reasoning to support the potential benefit of memantine in patients with ALS: 1) Memantine is an uncompetitive NMDA receptor antagonist that may reduce the effects of glutamate mediated excitotoxicity; 2) Memantine can inhibit and reverse the abnormal hyperphosphorylation of tau; 3) Memantine has been shown to prolong survival in a mutant SOD1 transgenic mouse model of ALS.

Objectives: 1) To see if ALS patients treated with a combination of rilutek and memantine progress more slowly than historical controls. 2) To follow levels of CSF biomarkers during the course of memantine treatment to explore whether changes correlate with response to treatment.

Methods: Twenty patients with ALS were enrolled in this open label pilot trial. Patients were treated with a combination of rilutek and memantine for 18 months. Lumbar punctures were performed at baseline, 6 and 12 months. CSF was analyzed in a blinded manner to assess specific biomarkers. ALSFRS was performed every three months.

Results: Compared to historical controls from other placebo controlled trials that lost an average of -0.90 points per month, the patients followed in this study for 18 months treated with memantine and rilutek lost on average -0.56 points per month. The levels of tau and neurofilament subunit (NF-H) at baseline were significantly higher in **our** patients with ALS compared to healthy controls. In addition, patients who progressed faster than -0.5 points per month had a higher levels of CSF tau and NF-H at baseline than those who lost less than -0.5 points per month.
Preliminary data have indicated that throughout the disease patients exhibit increased levels of CSF tau. In contrast patients in this trial showed a 27% decline in CSF tau after 12 months treatment. Further, patients whose CSF tau levels corrected back to that observed in healthy controls exhibited the slowest decline in ALS disease progression, reducing the rate of decline to -0.42 ALSFRS points per month.

Discussion: These data suggest that combination treatment with rilutek and memantine may slow down the disease course in patients with ALS. Further to this, higher levels of CSF tau and NF-H predict a faster rate of progression of the disease. Patients treated with memantine and rilutek who exhibit decreased levels of CSF tau over 12 months showed the most significant slowing of their disease progression.

Conclusion: The results of this open label pilot trial suggest that a double blind placebo controlled trial of rilutek and memantine is indicated. It is suggested that CSF tau and NF-H may be valid biomarkers for disease progression.

P14 RESULTS OF A RANDOMIZED CONTROLLED PHASE II TRIAL OF MEMANTINE FOR ALS.

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Keywords: memantine, clinical trial, neuroprotection

Background: There is sound evidence that glutamatergic excitoxicity is associated with motor neurons demise in ALS. Memantine is a novel class of Alzheimer's disease medication acting on the glutamatergic system by blocking NMDA glutamate receptors. This drug prolongs survival in an ALS mouse model (G93A) but there are no reports of clinical trials in human ALS.

Objectives: To report the detailed results of the clinical trial as an academic-funded, Phase II, double-blind, single-centre randomized, clinical trial of memantine 20 mg/day and Riluzole vs placebo and Riluzole.

Methods: Sixty-three patients who were treated for 12 months were studied. Inclusion criteria were 18–75 years of age, disease duration <36 months, probable or definite ALS (revised El Escorial criteria) and ALS-FRS score 25–38. Exclusion criteria were the presence of other medical or neurological diseases, FVC $<60\%$ predicted, ADM strength >2 bilaterally. Primary outcome was ALS-FRS change. Secondary outcomes were FVC, muscle force as evaluated by manual testing, analogue scale for disease progression, MUNE and NI of the ADM muscles. A questionnaire regarding side-effects was applied. The patients were evaluated at diagnosis, 1 month later, 3 months after entry and every 3 months thereafter for 12 months, Full evaluation was performed at entry, at 6 months and at one year. Number of patients needed to treat was calculated taking into account a 50% change in the ALS-FRS score (power 80%, $P<0.05$). Intention to treat and per-protocol analyses were performed.

Results: Both treatment and placebo groups were well balanced concerning age at onset, disease duration, gender, region of onset, ALS-FRS, FVC and body mass index. Drop-outs (8) and deaths (7) were equally distributed between groups. There was no difference between treatment groups in the primary or secondary outcomes measures at 6 months and after 12 months, with intention to treat or per-protocol analysis. Side-effects were comparable between groups.

Discussion: The effectiveness of Riluzole, an anti-glutamatergic drug, has raised the expectation that other

drugs acting in the same pathway could prove efficacious. Memantine is a licensed drug with neuroprotective action in Alzheimer's disease. This exploratory clinical trial in ALS was negative and does indicate that a larger Phase III is not promising.

Conclusions: The negativity if this trial suggests that modulating NMDA receptors by blocking glutamate transmission does not change the rate of motor neuron drop-out in ALS, in particular lower motor neurons.

P15 OXIDATIVE STRESS AND LITHIUM THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: lithium, oxidative stress

Background: The causes of motorneuron loss in Amyotrophic Lateral Sclerosis (ALS) are still unknown, but accumulating evidence indicates that oxidative stress is involved in the pathogenesis of this disease. The only treatment approved for this disease is Riluzole, an antiglutamatergic drug, that prolongs survival in ALS patients by about two months on average. Recently, a possible role of lithium salts for the treatment of this disease has been proposed. Lithium, a well known mood-stabilizing drug used for the treatment of bipolar affective disorders, has recently been recognized as a neuroprotective factor, mainly by its effects on cellular mechanisms linked to autophagy.

Objectives: To test the effect of lithium carbonate on clinical and oxidative-stress-related laboratory parameters of 30 sporadic ALS patients (mean $\pm$ SD age: 64.3 $\pm$ 7.0 years, mean $\pm$ SD duration of the disease 15.3 $\pm$ 9.4 months) through an open label therapeutic trial. The daily dosage of lithium has been targeted to the plasma levels ranging from 0.4 to 0.8 mEq/litre.

Methods: In the enrolled patients (20% bulbar, 30% cervical and 50% pseudopolyneuritic form), we assessed blood levels of advanced oxidation protein products (AOPP), ferric reducing ability of plasma (FRAP), total glutathione, as well as disease-related ALSFRS-r and MRC scales at baseline and at 6 months after the targeted plasma lithium level was achieved (T1 evaluation).

Results: A significant reduction of AOPP values (mean $\pm$ SD: 432.4 $\pm$ 279.8 vs 211.3 $\pm$ 163.1 μmol/L, P: 0.005) and increase of total glutathione (mean $\pm$ SD: 0.28 $\pm$ 0.13 vs 0.66 $\pm$ 0.33 nmol/μl of cells, P: 0.002) was observed, as compared to the values obtained before treatment. FRAP level was increased but not significantly (Mean $\pm$ SD: 0.52 $\pm$ 0.17 vs 0.83 $\pm$ 0.63 mmol/L Fe^{2+}-TPTZ, P: 0.08). Clinical evaluations at 6 months after the targeted plasma lithium level was achieved, showed a decrease of ALS-FRS and MRC scale scores compared to pre-treatment's scores.

Conclusion: Lithium therapy is able to reduce circulating levels of blood oxidative stress markers in ALS. Whether or not the modification of oxidative stress markers and antioxidant defence is related to a direct effect of this drug on the pathogenic mechanism of the disease is still an open question, to be addressed with long term studies in conjunction with the assessment of the clinical effects.

P16 SAFETY OF LITHIUM IN ALS PATIENTS

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Keywords: lithium carbonate, safety

Background: ALS is a fatal neurodegenerative motor neurone disorder leading to death within 3 years after onset. Recently, an open-randomized therapeutic trial suggested that ALS could be significantly slowed by lithium carbonate administered orally (1). This study generated enormous interest and many ALS patients requested lithium therapy despite its unknown efficacy and unclear safety in this population.

Objectives: To describe the clinical profile and outcome of ALS patients treated prospectively with lithium carbonate.

Methods: A total of 55 patients with ALS according to the El-Escorial criteria were treated with lithium at two centers. Group 1 included 28 patients treated in the US with IRB approval. Lithium was titrated on a weekly basis for the first month and then monthly, following the American Psychiatric Association practice guidelines. Group 2 included 27 patients in France where it is acceptable to treat ALS patients, providing the patients understand the risks and benefits of therapy. Contraindications were excluded, patients were followed quarterly and blood levels were monitored.

Results : In Group 1, 28 patients were treated with lithium. Five stopped prematurely (less than 3 months after lithium initiation) due to intolerance. Of 23 patients treated for a mean time of 6 months, 54% were male, mean age of onset was 56 $\pm$ 11 years, mean disease duration prior to lithium was 25 $\pm$ 16 months. Two patients died during treatment: One had a tracheostomy at 3 months and one died at 8 months. In Group 2, 27 patients were treated with lithium. Six stopped prematurely: two had intolerance; three perceived rapid worsening; and one suddenly died. Of 21 patients treated for a mean time of 6 months, 48% were male, mean age of symptom onset was 54 $\pm$ 12 years and mean disease duration prior to lithium was 36 $\pm$ 20 months. Four patients died during treatment within 7 months.
The rate of weight loss on lithium was greater (Group1: 0.49 kg/month; Group 2: 0.46 kg/month) compared to the rate of weight loss prior to lithium (Group 1: 0.01 kg/month, P = 0.04; Group 2: 0.04 kg/month, P = 0.02). The rate of ALSFRS decline after lithium treatment was also greater (Group 1: 1.5 points (pts)/month; Group 2: 0.95 pts/month) compared to prior to lithium (Group 1: 1.1 pts/month; Group 2: 0.64 pts/month).

Conclusion: The experience of lithium therapy among ALS patients in the centers was consistent and an increased weight loss on lithium therapy was noted. Weight loss cannot be excluded for being responsible for global worsening. Additionally, patients died while on lithium therapy which is different from that published previously. Worsening with lithium therapy should be considered, as it has already been observed in prior drugs that were thought to be promising. These data underline the need to strictly monitor lithium therapy. Only a well-designed randomized controlled trial can determine true efficacy and side effect profile.

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Acknowledgements: MDA, Wings Over Wall Street.

P17 CHRONIC LITHIUM DOSING IN A SIBLING-MATCHED, GENDER BALANCED, INVESTIGATOR-BLINDED TRIAL USING A STANDARD MOUSE MODEL OF FAMILIAL ALS HAS NO THERAPEUTIC BENEFIT

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Keywords: SOD1 G93A, therapy, lithium

In any animal model of human disease a positive control therapy that demonstrates efficacy in both the animal model and the human disease can validate the application of that animal model to the discovery of new therapeutics. Such a therapy has recently been reported using chronic lithium carbonate treatment (1) and demonstrated therapeutic efficacy in both the high-copy SOD1 G93A mouse model of familial ALS and in human patients. However, recent work by the Bendotti group did not identify any therapeutic or neuroprotective effect of lithium in SOD1 G93A female mice (2).

As part of an independent effort to identify a positive control therapy, we tested chronic lithium dosing in a sibling-matched, gender balanced, investigator-blinded trial using the high-copy (average 23 copies) SOD1 G93A mouse (n = 27–28/group). Lithium-treated mice received single daily 36.9 mg/kg i.p. injections from 50 days of age to death. This dose delivered 1 mEq/kg (6.94 mg/kg/day lithium ions). Neurological disease severity score and body weight were determined daily during the dosing period. Age at onset of definitive disease and survival duration were recorded. Summary measures from individual body weight changes and neurological score progression, age at disease onset and age at death were compared using Kaplan-Meier and Cox proportional hazards analysis. This study did not show lithium efficacy by any measure.

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P18 CHRONIC APOCYNIN DOSING IN A SIBLING-MATCHED, GENDER BALANCED, INVESTIGATOR-BLINDED TRIAL USING A STANDARD MOUSE MODEL OF FAMILIAL ALS PROVIDES NO THERAPEUTIC BENEFIT

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Keywords: SOD1 G93A, therapy, apocynin

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive paralysis due to motor neuron degeneration. Encouraging recent studies employing NOX knockout mice or treatment with a NOX inhibitor in the SOD1 G93A preclinical mouse model of ALS, concluded that dysregulated redox stress caused by NADPH oxidases Nox1 and Nox2 significantly influences the progression of motor neuron disease. The NOX inhibitor apocynin is a prodrug that is used to inhibit all members of the NOX family. Treatment of SOD1 G93A mice with the NOX inhibitor apocynin was shown to significantly prolong their average life span by approximately 4 months (1). Seeking to verify this as a positive control therapy in the SOD1 G93A mouse model, chronic apocynin dosing was tested at 150 mg/kg/day formulated in drinking water (an efficacious dosage level in the referenced study). Two sibling matched, gender balanced, investigator blinded trials were executed with two start ages, 30 days (n = 27/group) and 50 days (n = 26/group). The drug was determined to have been stable in its formulation throughout the study by LC/MS. Water consumption was measured twice weekly throughout the course of each study to confirm dosing estimates and rule out taste aversion to apocynin. Neurological disease severity score and body weight were determined daily during the dosing period. Age at onset of definitive disease and survival duration were recorded. Summary measures from individual body weight changes and neurological score progression, age at paresis onset and age at death were compared using Kaplan-Meier and Cox proportional hazards analyses.

Although the intended 150 mg/kg/day apocynin consumption occurred throughout the treatment period, neither treatment regimen showed apocynin efficacy by any measure in these rigorously powered, investigator-blinded, gender-balanced, sibling-matched survival studies.

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P19 A THERAPEUTIC AGENT (NDDPX08) DELAYS DISEASE PROGRESSION, IMPROVES MOTOR FUNCTION AND PROLONGS SURVIVAL IN A MOUSE MODEL OF ALS

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Keywords: oxidative stress, transgenic mouse, post-onset administration

Background: ALS is a devastating neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. Unfortunately, our understanding of the molecular pathogenesis of ALS is still limited. There is no perfectly validated molecular target in ALS. However, advancements in motor neuron disease research provide compelling evidence that oxidative stress is heavily implicated in neurodegenerative diseases including ALS. Neuronal apoptosis inhibitory protein (NAIP, aka BIRC1) selectively suppresses oxidative stress-induced cell death and the up-regulation and ectopic expression of NAIP exerts the neuroprotective activity in *in vivo* model of human cerebral ischemia. Based on the converging findings, NAIP is a potent molecular target in ALS drug discovery. A neurotropic compound library (1,000 chemicals) containing medical drugs was screened in NAIP-based drug screening. Among hit compounds, this study's interest converged on NDD-PX08, which is being used in another neurodegenerative

disease and has shown to selectively protect neuronal cell against oxidative insults *in vitro*.

Objectives: To conduct an efficacy test of NDDPX08 from disease onset in a congenic $SOD1^{H46R}$ mouse model of ALS, including neuropathological analysis.

Methods: Intraperitoneal injection of NDDPX08 (0, 1 and 10 mg/5 mL/kg) in 0.5% CMC-Na was conducted once a day starting at the day at which $SOD1^{H46R}$ mice exhibited signs of disease onset (post-onset administration). NDDPX08 efficacy in $SOD1^{H46R}$ mice was analyzed as follows; 1) behavioral testing (balance beam test, vertical pole test and footprint analysis) was performed once a week to assess motor function; 2) neuropathological analysis by immunohistochemical staining was performed by using the mice at 22 weeks of age.

Results: The post-onset administration of NDDPX08 extended survival interval of $SOD1^{H46R}$ mice after the sign of the onset compared to vehicle control, 39.0 ± 7.6 days (1 mg/kg, $n = 33$) and 39.4 ± 7.1 days (10 mg/kg, $n = 38$) vs. 35.2 ± 6.7 days (vehicle, $n = 31$), $P < 0.05$ by log-rank test. Behavioral analyses showed the preservation of motor function in the NDDPX08-treated mice when compared with the vehicle-treated mice. The treatment of NDDPX08 reduced the immunoreactivity for GFAP and Iba-1 in spinal cord of $SOD1^{H46R}$ mice. Furthermore, the delayed loss of ChAT-positive neurons in the anterior horn was observed in the NDDPX08-treated mice as compared with vehicle-treated mice.

Discussion and Conclusions: The post-onset treatment of NDDPX08 preserved the motor function and delayed the disease progression in a $SOD1^{H46R}$ mouse model. Thus, NDDPX08 is a highly promising candidate drug to the treatment of ALS. The clinical research of NDDPX08 as add-on to riluzole, a double-blind placebo-controlled trial with ALS patients, is being conducted.

Acknowledgements: This work was supported by the National Institute of Biomedical Innovation and the Ministry of Health, Labour and Welfare.

P20 PROOF-OF-CONCEPT FOR A NOVEL SMALL MOLECULE HSP90 INHIBITOR FOR SBMA

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Keywords: Hsp90, SBMA, neuroprotection

Background: A hallmark of motor neuron diseases (MNDs) and other neurodegenerative diseases is the misfolding and aggregation of mutant or otherwise mis-regulated proteins. In the case of spinal bulbar muscular atrophy (SBMA), a mutant poly-glutamine (poly-Q) expanded androgen receptor (AR) becomes mislocalized and aggregates to form inclusions. It has been shown that inhibition of the heat shock protein 90 (Hsp90) promotes the upregulation of multiple HSPs, the clearance of misfolded proteins and is neuroprotective in models for MNDs, including SBMA and other neurodegenerative diseases. Furthermore, the fact that AR is a client protein of Hsp90 provides additional rationale for Hsp90 inhibition as a therapeutic strategy for SBMA. NexGenix Pharmaceuticals is developing a series of fully synthetic small molecule Hsp90 inhibitors based on the natural radicicol scaffold. The lead compound, NXD30001, is a potent and relatively safe Hsp90 inhibitor which demonstrates *in vivo* efficacy in murine cancer models. In addition, NXD30001 readily penetrates the blood-brain barrier and accumulates in the brain, making it a viable therapeutic candidate for MNDs. This study hypothesized that NXD30001 will clear poly-Q AR aggregates in diseased motor neurons and will be efficacious in a drosophila model for SBMA.

Objectives: To develop an oral therapy for SBMA and MNDs based on the novel Hsp90 inhibitor series. This study's short-term objectives are to demonstrate efficacy for NXD30001 in a drosophila model for SBMA and to obtain data elucidating the mechanism(s) of protection by Hsp90 inhibition.

Methods: The efficacy of NXD30001 was evaluated in a drosophila model for SBMA that recapitulates many key features of human disease. The SBMA model also exhibits poly-Q length dependent impairment of ubiquitin proteasome function (1). In addition, the effects of NXD30001 on poly-Q AR localization and solubility as well as the validility of Hsp90 inhibition in the SBMA model both genetically and by assaying for biomarkers for inhibition was examined. The effects of NXD30001 in a conditional model for proteasome impairment was also investigated.

Results: NXD30001 treatment strongly suppressed neurodegeneration caused by expression of expanded poly-Q AR in a drosophila model of SBMA. Interestingly, NXD30001 rescued the degenerative phenotype in a dose dependent manner. NXD30001 treatment also rescued lethality caused by ubiquitous proteasome impairment in a drosophila model.

Discussion and Conclusions: These data provide evidence supporting the rationale for Hsp90 inhibition with NXD30001 in motor neuron degeneration associated with SBMA. Furthermore, the data presented here indicate a role for Hsp90 inhibition in suppressing neurodegeneration in the absence of a functional proteasome. Based on these results, pharmacologic inhibition of Hsp90 with NXD30001 is an attractive therapeutic strategy for SBMA and potentially other MNDs.

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P21 EVALUATING THE THERAPEUTIC POTENTIAL OF A NOVEL SMALL MOLECULE HSP90 INHIBITOR FOR MOTOR NEURON DISEASES

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Keywords: Hsp90 inhibitor, neuroprotection, heat shock proteins

Background: Heat shock proteins (HSPs) facilitate proper folding or clearance of misfolded proteins. Upregulation of multiple HSPs is protective in cell culture and transgenic mouse models of motor neuron diseases. Such treatments include the Hsp90 inhibitors, geldanamycin (GA) or 17-AAG, which increase expression of HSPs ostensibly through disrupting complexes of Hsp90 with factors involved in signaling the heat shock response and/or facilitating clearance. Toxicity

and poor penetrability of the blood-brain barrier limit therapeutic use of these agents in neurological diseases. In order to identify analogues with more favourable pharmacokinetic profiles, NexGenix Pharmaceuticals is developing a series of fully synthetic small molecule Hsp90 inhibitors based on the natural radicicol scaffold. The lead compound, NXD30001, is effective in the nanomolar range, exhibits greater safety factor in terms of induction of HSPs relative to cytotoxicity and accumulates in nervous tissue.

Objectives: To evaluate NXD30001 for its ability to induce HSPs, promote degradation of Hsp90 client proteins and confer neuroprotection in models of motor neuron disease.

Methods: Effects of NXD30001 on induction of HSPs (Hsp70, Hsp40 and Hsp90), turnover of Hsp90 client proteins and cytotoxicity were evaluated in BT474 breast cancer cells and in dissociated cultures of E13 mouse spinal cord-DRG, in comparison to other Hsp90 inhibitors (geldanamycin, 17-AAG and radicicol). Expression of each HSP was evaluated by Western blotting. Expression in neural cell types, motor neurons in particular, was evaluated by immunocytochemistry. Activation of the major heat shock transcription factor Hsf1 was determined by gel shift assay. Neuroprotective properties of NXD30001 are being investigated in primary culture models of familial ALS (cultured motor neurons expressing ALS-causing mutant proteins-including mutant SOD1) using multiple endpoints of toxicity (formation of inclusions, mitochondrial rounding, mitochondrial and cytosolic calcium and viability).

Results: NXD30001 depleted Hsp90 client proteins at lower concentrations than inducing Hsp70 expression in BT474 cells. In spinal cord-DRG cultures, NXD30001, GA and 17-AAG induced dose-dependent expression of Hsp70 and Hsp40, but not Hsp90 at non-cytotoxic concentrations. NXD30001 induced expression of Hsp70 predominantly in motor neurons, whereas Hsp40 was upregulated in a larger number of cells. Hsf1 hyperphosphorylation, indicative of activation, was increased in a dose-responsive manner.

Discussion and Conclusions: Radicicol, the parent compound of NXD30001 transiently induced HSPs in cultured motor neurons, but only at cytotoxic concentrations. NXD30001 induced more sustained expression of multiple HSPs and was less cytotoxic. Hsp90 inhibitors show promise in therapy of disorders of protein misfolding including ALS-GA is highly neuroprotective in this primary culture model of fALS1. Due to its more favourable pharmacological profile and ability to accumulate in nervous tissue, NXD30001 is an attractive candidate for testing in animal models and for therapeutic development.

P22 SOD1G93A MICE TREATED WITH A SOLUBLE FORM OF THE ACTIVIN RECEPTOR TYPE IIB (ACTRIIB) HAVE INCREASED LEAN MUSCLE MASS AND IMPROVED GRIP STRENGTH

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Keywords: myostatin, muscle hypertrophy, activin receptor type IIB

Background: Amyotrophic lateral sclerosis (ALS) is characterized by progressive loss of muscle mass, strength and mobility due to the deterioration of motor neurons. $SOD1^{G93A}$ mice express a mutant form of the human Cu/Zn superoxide dismutase (SOD1) gene and display progressive muscle weakness, muscle atrophy and paralysis similar to ALS. Activin receptor type IIB (ActRIIB) mediates the effects of multiple ligands of the TGF-beta superfamily which act as negative regulators of muscle mass. Administering RAP-031, a fusion protein derived from a form of the extracellular region of ActRIIB fused to an IgG Fc domain, prevents signalling of these ligands resulting in dramatic increases of muscle mass and strength.

Objectives: To evaluate the effects of RAP-031 on muscle mass and strength in $SOD1^{G93A}$ mice with advanced disease.

Methods: 125-day old B6.Cg-Tg($SOD1^{G93A}$)1Gur/J mice, a $SOD1^{G93A}$ strain maintained on the C57BL/6 background with a life expectancy of approximately 160 days, were divided into male and female groups that received either control vehicle or RAP-031 twice-a-week for 3 weeks. NMR scans were performed at baseline (125 days of age) and study end to assess body composition. Forelimb grip strength was measured at baseline and 143 days of age.

Results: At study end, control group male mice lost 8.3% of body weight (day 125: 25.4 ± 1.3 g, day 146: 23.3 ± 1.2 g $P < 0.001$); however, the body weight of RAP-031-treated males did not change significantly compared to baseline (day 125: 25.7 ± 1.2g, day 146: 26.2 ± 1.7g P = not significant (ns)). In female mice, body weight of control animals did not change significantly (day 125: 18.8 ± 1.0 g, day 146: 18.7 ± 0.9 g P = ns); however, RAP-031 treatment resulted in a 12% increase in body weight (day 125: 19.1 ± 1.0 g, day 146: 21.4 ± 1.2 g $P < 0.001$). The different effects on body weight of RAP-031 and control animals were attributable to changes in lean mass, as assessed by NMR scans. At 146 days of age, RAP-031-treated male and female mice had 11.5% and 14.5% more lean tissue mass, respectively, compared to vehicle-treated mice ($P < 0.01$). Treatment with RAP-031 also mitigates loss of motor function, as assessed by forelimb grip strength. Male control animals lost significantly more grip strength (-39.6%) than RAP-031 treated animals (-18.6%) in the day 143 trial compared to the baseline grip strength trial. Similarly, female control animals lost significantly more grip strength (-34.0%) compared to RAP-031 treated animals (-21.6%) across the two trials.

Discussion and Conclusions: These data demonstrate that RAP-031 increases body weight, lean tissue mass and improves muscle function in late-stage $SOD1^{G93A}$ mice. Therefore, treatment with ACE-031, the fully human analogue of RAP-031, could provide clinical benefit for ALS patients with advanced disease. A Phase I clinical study of ACE-031 is currently underway.

P23 NEUROPROTECTIVE ACTIVITY OF NOVEL MULTIMODAL IRON CHELATING DRUGS IN MOTOR NEURON-LIKE NSC-34 CELLS AND TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: multimodal iron chelating drugs, hypoxia-inducible factor, G93A-SOD1 mouse model

Background: Amyotrophic Lateral Sclerosis (ALS) is a multifactorial disease where several pathological mechanisms

have been suggested to contribute to neurodegeneration. Novel therapeutic approaches for the treatment of ALS may, therefore, consider drug candidates designed specifically to act on multiple central nervous system targets. Multi-functional, non-toxic, brain-permeable iron chelating drugs, M30 and HLA20, possessing the N-propargylamine neuroprotective moiety of the anti-Parkinson's drug, rasagiline (Azilect) and the iron-chelating moiety of VK28 have recently been synthesized.

Objectives: To examine the neuroprotective activities of our multifunctional drugs in the motor neuron-like cell line NSC-34 and transgenic mouse model of ALS.

Methods: The protective action of M30 and HLA20 were investigated in NSC-34 naïve and NSC-34 expressing mutant G93A-SOD1 protein cell lines. The effects of M30 and HLA20 on protein and mRNA levels of apoptosis/ survival-related markers and signaling pathways were examined by Western immunoblotting, RT-PCR and immunohistochemical analyses. To investigate the effect of M30 *in viv*o, G93A-SOD1 transgenic mice were randomly assigned to vehicle or M30. M30 (1mg/kg) and vehicle were administered by oral gavage method, four times a week, starting at 70 days of age. Various behavioral tests were performed to assess the effects of the drugs on the onset and extent of neurological deficits.

Results: M30 and HLA20 possess a wide range of pharmacological activities in mouse NSC-34 motor neuron cells, including neuroprotective effects against hydrogen peroxide- and 3-morpholinosydnonimine (SIN-1)-induced neurotoxicity; induction of differentiation and up-regulation of hypoxia-inducible factor (HIF)-1α and HIF-target genes (enolase1 and vascular endothelial growth factor). Both compounds induced NSC-34 neuritogenesis, accompanied by a marked increase in the expression of brain derived neurotrophic factor (BDNF) and growth associated protein (GAP)-43, which was inhibited by PD98059 and GF109203X, indicating the involvement of mitogen-activated protein kinase (MAPK)/ERK kinase (MEK) and protein kinase C (PKC) pathways, respectively. A major finding was the ability of M30 to significantly extend the survival of G93A-SOD1 ALS mice and delay the onset of the disease.

Discussion: The neuroprotective/neuro-rescue potential of the novel compounds may result from their multifunctional activities: a) similar to rasagiline and other propargyl-containing molecules they activate the canonical survival pathways, MEK and PKC, associated with elevation of BDNF; b) the promotion of neurite sprouting may result from activation of the above pathways, combined with the ability of iron-complexing molecules to interfere with cell cycle progression via deactivating cell cycle regulators, thus triggering differentiation through various iron-associated biological events; c) activation of HIF-1 and induction of its pro-survival/ neuroprotective target genes, an action that has been ascribed to iron chelation and inhibiton of HIF prolyl 4-hydroxylases (PHDs).

Conclusions: These data indicate that these new multi-functional molecules may have the potential to modify the progression of ALS.

P24 OVEREXPRESSION OF G-CSF PROTECTS MOTONEURONS AGAINST APOPTOTIC DEATH IN PERIPHERAL NERVE AXOTOMY OF NEONATAL MICE

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Keywords: G-CSF, axotomy, neonatal

Granulocyte colony stimulating factor (G-CSF) is known as a hematopoietic growth factor that is essential for generation of neutrophilic granulocytes. Potent neuroprotective and regenerative properties of G-CSF in the CNS have recently been uncovered. The G-CSF receptor and G-CSF itself are expressed in large motoneurons, G-CSF protects motoneuronal cell lines and improves outcome in the SOD1 (G93A)-transgenic mouse model for amyotrophic lateral sclerosis (ALS). Due to the pleiotrophic effects of G-CSF it is not fully clear at present if the effects seen in ALS models are solely due to motoneuron-specific effects, or if other components are also important.

This study sought to further delineate the anti-apoptotic potential of G-CSF on motoneurons *in vivo* in a different model, sciatic nerve axotomy in newborn mice. The sciatic nerve axotomy model was used in neonatal mice overexpressing G-CSF in the CNS. This is a well established experimental paradigm of motoneuron death. This study found that G-CSF transgenic animals displayed higher numbers of surviving lumbar motoneurons 4 days following axotomy.

In this model of pure apoptotic cell death, the protective effects of G-CSF indicate direct actions of G-CSF on motoneurons *in vivo*. This strengthens the argument for activity of this direct mechanism of action also in complex motoneuron disease models like the SOD1 (G93A) transgenic mouse.

P25 RESCUE OF MOTOR NEURONS IN ALS BY TARGETING THE BNIP3 CELL DEATH PATHWAY

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Keywords: mitochondria, oxidative stress, BNIP3

Oxidative stress, mitochondrial dysfunction and morphologically necrotic-like motor neuron death are major features in ALS. Previously it has been shown that oxidative stress provides a redox signal to activate hypoxia-inducible factor 1α (HIF-1α), which is the primary, if not the only, transcriptional factor for the death-inducing gene BNIP3 (1). Expression of BNIP3 caused a caspase-independent form of neuronal cell death *in vitro* and *in vivo* (2).

Here it is shown that BNIP3 expression was inducted at the onset of the disease in transgenic mice expressing the G93A and the G37R mutations of SOD1. BNIP3 was not detectable in the brain of control animals nor in the G93A or G37R mice before the onset of disease. Levels of BNIP3 expression increased with disease progression as determined by immunohistochemistry, Western blotting and RT-PCR analyses. The expressed BNIP3 was found to be primarily localized to

motor neurons. BNIP3 was not detectable in the liver, kidney and lung tissues from the same groups of G93A and G37R animals that showed high levels of BNIP3 in the spinal cord. BNIP3 was detected in the mitochondrial membranes after alkaline extraction, indicating that the expressed BNIP3 was active because inactive BNIP3 is known to be dissociated from mitochondria after alkaline treatment.
To further determine the role of BNIP3 in mutant SOD1-induced neuronal death, a lentiviral shRNA vector targeting the nucleotides 167–188 of the BNIP3 mRNA, which was able to almost completely inhibit BNIP3 expression (2), was injected into the lumbar spinal cord of the G93A mice at the age of 8 weeks. Animals injected with a scramble shRNA vector were used as controls. Inhibition of BNIP3 by RNAi significantly increased the number of axons in the L5 ventral roots ($p = 0.015$). Analysis of axon size distribution showed clearly the protection of middle to large (larger than 6 mm in inner diameter) axons by the lentiviral BNIP3 shRNA vector. The BNIP3 pathway was further analysed and found that BNIP3 interacted with the ion channel VDAC to induce mitochondrial release of endonuclease G leading to a caspase-independent apoptosis. To look for an inhibitor for the BNIP3 pathway, the small chemical necrostatin-1 was identified that was able to inhibit BNIP3 cell death pathway by preventing integration of BNIP3 to the outer membrane of mitochondria. The results demonstrate that BNIP3 plays a role in mediating mutant SOD1-induced motor neuron death. The BNIP3-induced cell death pathway provides a molecular linkage for mitochondrial degeneration, oxidative stress and caspase-independent neuronal death. Necrostatin-1 appears to be a potent inhibitor for the BNIP3 pathway and may be a new therapy for ALS.

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P26 LIF DECREASES AMPA RECEPTORS IN THE BRAINSTEM AND SENSORIMOTOR CORTEX OF SOD1G93A(G1H) MOUSE MODEL OF ALS

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Keywords: AMPA, LIF

Background: Motor neurons degenerate in patients with amyotrophic lateral sclerosis (ALS) and the mechanism of this disease is still unclear. Endogenous neural stem cells (ENSCs) are considered to be a promising way of treating ALS in the future.

Objectives: To observe the expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the brainstem and sensorimotor cortex of the $SOD1^{G93A}$ (SOD1) transgenic mouse model of ALS and identify whether leukemia inhibitory factor (LIF) can reduce the expression and inhibit the neurotoxicity of AMPA receptors.

Methods: Fourty-eight transgenic mice resourced from B6SJL-TgN (SOD1-G93A) which expressed a human mutation in the superoxide dismutase 1 gene were used in this study. These mice were separated into 3 groups; ALS control (ALSC); LIF treated (LIF) and normal control (CON) group each group contained 16 mice (8 males and 8 females). Radioligand binding assays were carried out in this experiment to estimate the expression of AMPA receptors in brainstem and sensorimotor cortex in each of the three groups. The number of AMPA receptors was calculated using the confocal counting system.

Results: A total of 48 mice were involved in the result analysis. 1) Compared with the CON, at the time points of postnatal 90-day and 120-day, the number of AMPA receptors on the motor neurons of brainstem increased to 1.86 and 3.30 times in the ALSC group (both $P < 0.01$), 1.16 times ($P > 0.05$) and 2.48 times ($P < 0.01$) in the LIF group, respectively. Compared with the ALSC group, the expression of AMPA receptors in brainstem and sensorimotor cortex in the LIF group decreased significantly ($P < 0.01$). 2) The number of AMPA receptors on the motor neurons of sensorimotor cortex increased to 1.71 times and 4.95 times in the ALSC group (both $P < 0.01$), while 1.18 times ($P > 0.05$) and 3.35 times ($P < 0.01$) in the LIF group, respectively at days 90 and 120 after birth. The expression of AMPA receptors decreased in the sensorimotor cortex in the LIF group compared with the ALSC group ($P < 0.01$).

Discussion: AMPA is a glutamate receptor found on the postsynaptic membrane and is related to the activity of excitotoxic glutamate. In this study the number of AMPA receptors in an ALS model increased at the late stage of disease, which may contribute to the loss of neurobehavior function. Further to this, the number of AMPA receptors was also found to be decreased in the LIF treated animal model, this mechanism needs to be further investigated.

Conclusion: The number of AMPA receptors in the brainstem and sensorimotor cortex of ALS mice significantly enhances at the middle and late stages of this disease, which is associated with glutamate-mediated toxicity of motor neurons.

P27 MULTIPLE ADMINISTRATIONS OF HUMAN MARROW STROMAL CELLS THROUGH CEREBROSPINAL FLUID MAY PROLONG SURVIVAL OF SOD1-G93A MICE

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Keywords: SOD1 G93A mice, marrow stromal cells, cerebrospinal fluid

Background: The blood-brain barrier (BBB) is the main obstacle for cell therapy in neurological disorders such as amyotrophic lateral sclerosis (ALS). Intrathecal injection is a potential method for cell transplantation because it would bypass the BBB. We investigated the effects of human marrow stromal cells (hMSC) delivered through cerebrospinal fluid (CSF) in SOD1-G93A mice, a transgenic mouse model of ALS.

Methods: 5×10^5 hMSC were delivered into the CSF of SOD1-G93A transgenic mice at the age of 8 weeks (single transplantation group) or 8, 10 and 12 weeks (multiple transplantation group). Clinical features, weight, hanging wire test and motor neuron count were observed to assess disease progression in the SOD1-G93A mice. Immunohistochemistry was performed with a human-specific antibody against HuNu to measure the distribution of hMSC in the

lumbar spinal cord parenchyma of SOD1-G93A mice at the age of 15 weeks.

Results: A single transplantation of hMSC did not have a beneficial effect in SOD1-G93A mice. Multiple transplantations of hMSC attenuated weight loss, enhanced motor performance, decreased motor neuron loss and, importantly, increased survival in SOD1 transgenic mice. However, only a few hMSC delivered through the CSF migrated into the lumbar spinal cord parenchyma of SOD1 mice.

Conclusions: Multiple administrations of hMSC through CSF may have a therapeutic effect in SOD1-G93A mice, although limited numbers of cells migrate into the lumbar spinal cord parenchyma. It is likely that the hMSC remaining in CSF are responsible for the effect in SOD1-G93A mice.

P28 CELLULAR FATES OF SYSTEMICALLY DELIVERED NEURAL STEM CELLS IN A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: neural stem cells, cell fates, cell transplantation therapy

Background: One of the common drawbacks of focal injections of stem cells in ALS experimental models is the limited number of regions which can be targeted by applying such procedure.

Objectives: As ALS is defined by widely spread pathogenetic processes affecting the motor cortex and the spinal cord, it was of high interest to test whether blood delivery route could yield satisfactory level of cell survival and differentiation. Herewith, we report our work with neural stem cell (NSC) therapy of ALS rat animal model (G93A).

Methods: In order to obtain an insight into cellular fates after blood stream injection, we developed a specific protocol: it included isolation of NSC from the cortex of 16 day old rat embryos ubiquitously expressing GFP, cultivation of neurospheres and obtaining of uniform 100% nestin/GFP positive population of NSC.

Results: Injection of GFP positive NSC into the blood stream of affected animals resulted in the presence of NSC within the affected CNS. The cell amount found within their wild type counterparts was significantly lower. NSC remained in an undifferentiated state for the first day, but after 3 days started to differentiate: approximately 35% were GFAP positive (astrocytes) and 25% were Map2 positive (neurons). The remaining 40% cells were undifferentiated (30%) or expressed other markers including O4 (oligodendrocytes) and Iba1 (microglia). Analyses of programmed cell death using a caspase specific assay revealed that approximately 35% of Map2+ cells and less than 15% of GFAP+ cells died within 7 days after injection possibly caused by failure of cell integration within the host tissue. However, after 14 days, the remaining Map2+ and GFAP+ cells continue to be present in the motor cortex.

Conclusions: Prolonged survival of injected NSC cells and the presence of synaptic marker synaptotagmin reveal that transplanted cells can survive and integrate within disease affected regions. Thus, these results suggest that systemic route for delivery of NSC in ALS affected CNS represents a promising strategy for cell transplantation therapy of ALS.

P29 EVALUATION OF THE THERAPEUTIC POTENTIAL OF INTRASPINAL TRANSPLANTATION OF HUMAN UMBILICAL CORD BLOOD CELLS IN G93A ALS MICE

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Keywords: adult stem cells, G93A mice, neuroprotection

Background: Cellular therapies appear to be a promising option for both familial and sporadic ALS even though there are still major limitations to overcome. The focus of cell therapies in ALS currently lies in the generation of a neuroprotective environment for degenerating motor neurons by transplantation of non-neuronal cells. Adult stem cells from umbilical cord blood present an option worth further investigations as they are barely immunogenic, ethically inoffensive, non-tumorigenic and easily available. Despite their hematopoetic origin, human umbilical cord blood cells (hUCBC) were shown to differentiate *in vitro* along neuronal or glial lineages.

Methods: G93A SOD1 transgenic mice received intraspinal injections of hUCBCs positive for CD34+ and expanded in presence of stem cell growth factors for 8 days before (d40) or after symptom onset (d90). Lentiviral eGFP-labeling was used for post mortem detection of transplanted cells by fluorescence microscopy. For *in vivo* detection by MRI, cells were labeled with iron oxide nanoparticles. Survival time of G93A-ALS transgenic mice transplanted with either hUBCB or vehicle before or after symptom onset was assessed. The locomotor activity was determined weekly via rotarod and footprint analyses. In addition, immunohistological and molecular biological evaluation of spinal cord tissue was performed.

Results: With the aid of a stereotactic frame a reproducible and defined administration of cells into the affected region was found to be possible and the blood brain barrier can be overcome. Detection of transplanted cells was also possible both post mortem and *in vivo.*

Discussion: For the most efficient administration of stem cells for the treatment of ALS, a local injection of the cells appears to be plausible. Intraspinal transplantation into the lumbar spinal cord does not cause persisting motor impairment or major tissue lesions. Before translation into clinical trials can be considered, basic questions need to be clarified, such as migration, functional state and interaction with the environment of the transplanted cells *in vivo.*

P30 ACETYLCHOLINESTERASE IS OVEREXPRESSED IN ALS MICE AND ANTISENSE INHIBITION PROLONGS SURVIVAL

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Keywords: antisense oligonucleotides, acetylcholinesterase, mouse model

Background: Acetylcholinesterase (AChE) may be involved in ALS through various mechanisms, including exacerbation

of excitotoxicity and accelerated apoptosis. Most deleterious effects of AChE are unrelated to its enzymic activity. AChE enzyme inhibitors such as neostigmine are not candidate treatments in ALS as they upregulate a deleterious form of AChE known as "read-through" AChE (AChE-R). AChE antisense oligonucleotides (EN101) decrease AChE-R levels in muscle and spinal-cord neurons and alleviate symptoms of myasthenia gravis in humans. The beneficial clinical effects of EN101 in ALS mice have previously been described.

Objective: To further investigate the role of AChE-R in ALS by checking AChE-R levels in ALS mice. To investigate the clinical, pathological and biochemical effect of EN101 on ALS mice when compared to treatment with neostigmine.

Design and Methods: 1) Untreated G93A ALS mice and wildtype (WT) mice were sacrificed for comparison of AChE-R levels in spinal neurons (3 mice per group). 2) ALS mice received either saline (NS, n = 35), EN101(n = 35), inverse-EN101(n = 15) or neostigmine (n = 15). Treatments were administered daily from 5-weeks (oligonucleotide dose = 200 μg/kg, neostigmine dose = 1 mg/kg). 3) disease-onset and survival were recorded. Additional mice (7 from the EN101 group and 3 from the NS group) were sacrificed for motor-neuron count and AChE-R level measurement (quantitative ELISA).

Results: AChE-R staining density was significantly higher in G93A mice compared to WT (80μ vs 140μ, $P < 0.01$). Compared to NS, EN101 resulted in a non-significant 5-day delay in disease onset and a 9-day delay in death ($P < 0.001$). Neostigmine treatment resulted in earlier onset (5 days, $P < 0.05$) and death (6 days, non-significant). No significant clinical effects were observed with inverse EN101. Mice treated with EN101 had significantly higher cell-counts at 15 weeks of age compared to untreated mice. AChE-R staining density was significantly lower in EN101 treated ALS mice compared to untreated ALS mice. (110μ vs 140μ, $P < 0.01$).

Conclusions and Relevance: High AChE-R levels in ALS mice supports the role of AChE-R in ALS. Treatment with EN101 reduces AChE-R expression in ALS mice to levels found in WT mice. The beneficial effects of EN101 coupled with the detrimental effects of neostigmine (which raises AChE-R) further supports the role of AChE-R in ALS.

P31 POTENTIAL NANOTECHNOLOGY PLATFORM FOR ALS: EFFICACY IN THE MSOD1G93A MOUSE MODEL

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Keywords: nanotechnology, animal model, inflammation

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease hallmarked by the selective death of motor neurons accompanied by inflammation and microgliosis, the latter of which has been shown to have a neurotoxic role in the pathogenesis of the disease. To this end, several therapeutic agents have aimed to curb microglia activation, albeit with little success. To increase site-specific concentration and thus drug efficacy, a liposomal drug delivery system with targeting moieties specific to microglial cell surface markers was utilized to deliver the anti-inflammatory compound minocycline.

Objectives: To develop a targeted nanotechnology platform that will improve treatment options for individuals suffering from ALS. To address this objective we provide a proof of concept study using minocycline contained in liposomes and delivered via intracerebroventricular (ICV) pump to mice carrying the Cu/Zn superoxide dismutase-1 (SOD1) mutation.

Methods: To test these hypotheses, the liposomes were conjugated with an LPS targeting moiety that is recognized by the Toll4 receptors on microglia. The drug was delivered continuously via an ICV osmotic pump that was implanted at 70 days of age. Control animals had a pump inserted that delivered only a buffer. Efficacy was determined by the time to disease onset in treatment versus control groups, as well as the time to endpoint stages. Motor performance was measured with a rotarod apparatus with disease onset defined as the point in which a mouse can no longer stay on the rotating rod for >1 s.e.m. of the number of times it fell during the pre-symptomatic phase.

Results: Control mice have an average disease onset at 107 days of age, whereas mice receiving targeted and non-targeted minocycline liposomes have an average disease onset at 113.4 ($P < 0.05$) and 118.3 days ($P < 0.001$), respectively. Targeting the nanovesicles to microglia appears to significantly slow later disease progression.

Discussion and Conclusions: In the present study, both age of disease onset and lifespan are significantly increased with minocycline liposomal treatments. These effects were obtained with concentrations that were lower than those given in previous studies using intraperitoneal or oral routes of delivery. This is a consistent advantage of delivering therapeutic agents with a nanotechnology platform. Minocycline has not been successful in treating ALS in humans. However, this study serves as a proof of concept for a drug delivery platform in which the "payload" of the nanovesicles can be easily modified to other compounds, including cDNA or siRNA, which could be delivered via an intraventricular route with the goal of altering the disease course of ALS.

P32 INVESTIGATING NOVEL ROUTES OF DELIVERY FOR GENE THERAPY APPROACHES TO ALS

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Keywords: gene therapy, lentivector, VEGF

Background: It has previously been shown that delivery of vascular endothelial growth factor (VEGF) using a retrogradely transported EIAV lentiviral vector pseudotyped with a rabies-G envelope protein (MoNuDin®) prolongs survival and delays motor neuron degeneration in a mouse model of ALS ($SOD1^{G93A}$) following intramuscular delivery. Clinical advancement of this approach has been limited by the necessity to administer large amounts of vector to many muscle groups. For these reasons alternative routes of administration are currently being investigated with the aim of maximising delivery of VEGF to motor neurons and minimising the dose of vector required.

Objective: Identification of novel routes of delivery for therapeutic application of MoNuDin.

Methods: A bicistronic EIAV vector encoding the human VEGF and the reporter gene GFP (EIAV-VEGF-GFP) was constructed to allow visualisation of transduced cells. The vector was administered using several routes of delivery including intraspinal, intraventricular and direct injection into the deep cerebellar nucleus (DCN). Transgene expression was evaluated in target tissues at one month post vector delivery.

Results: Intraventricular delivery of EIAV-VEGF-GFP vector pseudotyped with the VSV-G envelope protein resulted in some transduction of the ependymal cell layer lining the ventricular system as assessed by GFP immunohistochemistry. Direct delivery of an EIAV vector pseudotyped with a rabies-G envelope protein to the DCN resulted in efficient retrograde transport of vector to several brain stem nuclei, including the ventral tegmental nucleus, inferior olive and medial nuclei. Assessment of vector transduction and transgene expression following direct intraspinal delivery is under investigation.

Discussion and Conclusions: The intraventricular delivery route provides an interesting alternative site of delivery for MoNuDin. Although modest transduction efficiency was achieved in this study, improvement may be made by altering the pseudotype of the EIAV vector to confer a higher tropism for the ependyma and by increasing vector dose.
Although the brain stem nuclei transduced by DCN delivery are not necessarily those most relevant for ALS therapy, the secreted VEGF protein would be likely to spread from the sites of transduction to the motor neuron populations of the brain stem. The results of the intraspinal delivery of EIAV vectors will also be presented at the meeting.

P33 GENE THERAPY APPROACHES TO NEUROPROTECTION IN EXPERIMENTAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: gene therapy, lentiviral vectors, anti-oxidant genes

Background: Amyotrophic lateral sclerosis (ALS), is a progressive neurodegenerative disorder characterized by the loss of upper and lower motor neurons with no effective treatment to date. Although it has a multi-factorial aetiology, oxidative stress is hypothesized to be one of the key pathogenic mechanisms since in cellular models of ALS decreased expression of anti-oxidant genes and disrupted anti-oxidant defence in mitochondria has been observed.

Objectives: It has been previously found that three specific anti-oxidant genes, namely peroxiredoxin 3, leukotriene B4, 12-hydroxydehydrogenase and nuclear factor erythroid 2-related factor 2 (Prdx3, LTB412HD and NRF2), are down-regulated in the presence of mutant SOD1 (mutated in approximately 5% of ALS cases). The main aim of this project is to alter the expression of one or more target genes using viral vectors and study the effect on the vulnerability of cells expressing mutant SOD1 both *in vitro* and *in vivo*.

Methods: Two different cellular models (NSC34 cell line or NSC34 carrying a common mutation in SOD1–G93A mutation and mouse primary motor neurons) were used in order to achieve the over-expression of the target genes using a lentiviral delivery system. After verification of the target gene over-expression through western blotting and Q-PCR, the oxidation stress levels were measured using 2′,7′-dichlorofluorescein (DCF) dye. Cell survival of NSC34 cells was assessed by 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay.

Results: The cells transduced with the lentiviral vectors expressing the anti-oxidant genes achieved stable over-expression of the target proteins and preliminary data showed promising neuroprotection properties of most of the genes. In particular, wild type NSC34 cells transduced with lentiviral vectors expressing $LTB_4$12HD showed no neuroprotection after inducing oxidative stress and this gene was not studied any further. When NSC34 cells were transduced with viral vectors expressing NRF2, over-expression of NRF2 was not stable over time possibly due to tight regulation with maximum over-expression seen only 2 days post transduction. However, when NSC34 G93A cells were transduced, the over-expression remained stable for at least 10 days and a 60% reduction in oxidative stress was seen ($N = 3$, $P < 0.001$). Finally, NSC34 cells transduced with lentiviral vectors expressing Prdx3 exhibited a consistent 30% protection against induced oxidative stress (serum withdrawal or menadione, $N = 5$, $P < 0.01$) and 40% reduction of oxidative stress in NSC34 cells carrying the G93A SOD1 mutation at the basal level ($N = 5$, $P < 0.001$). The neuroprotective properties of Prdx3 are currently being verified by cell viability assays in mouse primary neurons.

Discussion and Conclusions: The lentiviral delivery of anti-oxidant genes is proven to be an attractive strategy to protect motor neurons long term. Thus, the most promising gene (Prdx3) will be taken forward to the animal model of ALS, with ultimate aim the translation of this strategy to the clinic.

P34 INTRAVENOUS OPTIMIZED SCAAV9 VECTORS FOR TRANSGENE DELIVERY TO ADULT MOTOR NEURONS.

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Keywords: adeno-associated vector, gene therapy, motor neuron

Motor neuron diseases (MNDs) such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are neurodegenerative disorders of the central nervous system (CNS) characterized by motor neuron (MN) degeneration. To date, there is no treatment for these disorders mainly due to the blood brain barrier (BBB) which prevents drug diffusion into the CNS. Gene transfer to the spinal cord based on intramuscular injections of viral vectors has been successfully used in laboratory animals to produce therapeutic proteins within MNs. However, this strategy is clinically difficult because of the size and the number of muscles that should be targeted in the whole human body.
Here the potential for MN transduction of an alternative gene therapy strategy was analysed, based on the intravenous administration of recombinant vectors derived from the adeno-associated-virus of serotype 9 (scAAV9). Single stranded (ss) and double-stranded self-complementary (sc) AAV1 and 9 vectors were injected intraperiteonaly, intramuscularly or intravenously in neonatal or adult mice and analyzed transgene expression in the CNS. These vectors encoded either the murine secreted alkaline phosphatase (mSEAP) or the green

fluorescent protein (GFP) under control of the cytomegalovirus (CMV) promoter. These results showed, for the first time, that intravenous scAAV9 enabled widespread, non-invasive and long-term MN transduction in adult mice, without pharmacological disruption of the BBB.
As the scAAV9 vectors used in this study expressed the reporter gene under control of the CMV promoter, systemic vector-mediated transduction was not restricted to MNs. This could yield severe side effects due to undesired transgene expression (for example, trophic factors) in tissues such as the heart or the liver. We thus proposed to optimize the scAAV9 vectors in order to restrict transgene expression to neurons following intravenous injection, thereby reducing possible side effects due to ectopic transgene expression. In this aim, the study used and compared two strategies: (i) the use of the Synapsin neurospecific promoter (ii) the use of Neuron-Restrictive Silencer Elements (NRSEs) placed upstream from the ubiquitous phosphoglycerate promoter. The potential of these new scAAV9 vectors for achieving high and specific transgene expression in the CNS was assessed both in culture and in mouse models of MND.

P35 FRAGMENT C OF TETANUS TOXIN, MORE THAN A CARRIER: NOVEL PERSPECTIVES IN NON-VIRAL ALS GENE THERAPY

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Keywords: SOD1 (G93A) mice, fragment C of tetanus toxin, non-viral gene therapy.

Background: The non-toxic carboxy-terminal fragment of tetanus toxin heavy chain (TTC) has been implicated in the activation of cascades responsible for trophic actions and neuroprotection by inhibition of apoptosis (1,2). In particular, in Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative disorder involving the loss of cortex, brainstem and spinal cord motor neurons, TTC may act as a potential therapeutic agent. TTC is sufficient for neuron binding, internalization, retrograde and transynaptic transport (3), thus becoming an attractive and promising therapeutic agent.

Objectives: To investigate the effects of intramuscular delivery of a TTC-encoding plasmid in a neurodegenerative mouse model. To analyze clinical features and focus on gene expression and protein assays to detect anti-apoptotic processes in the spinal cord of one of the best characterized ALS mouse models $SOD1^{G93A}$ mice.

Methods: Naked DNA encoding for TTC was injected intramuscularly into the quadriceps and triceps muscles in $SOD1^{G93A}$ mouse model, neuromuscular function and clinical behaviours were monitored until end stage. Gene expression variations in spinal cord and muscle tissues due to TTC treatment were assayed by real-time PCR. Protein expression variations in spinal cord due to TTC treatment were assayed by Western blot analysis. Statistical significance was evaluated by one- or two-factor ANOVA and posterior Post-hoc analysis.

Results: These results indicate that TTC treatment ameliorated the decline of hindlimb muscle innervation, significantly delayed the onset of symptoms and functional deficits, improved spinal motor neuron survival and prolonged lifespan by 12.8 days in the TTC-treated group compared to the vehicle-plasmid group. Furthermore, caspase-1 and caspase-3 proapoptotic genes were down-regulated in the spinal cord of treated mice. Western blot analysis showed that the active form of caspase-3 was also down-regulated. Survival signals, such as Akt phosphorylation, were also up-regulated after TTC treatment compared to vehicle-plasmid mice. TTC treatment revealed variations in calcium-related gene expression (Ncs1 and Rrad) in spinal cords of TTC-treated $SOD1^{G93A}$ animals.

Discussion and Conclusions: These results suggest that fragment C of tetanus toxin, TTC, provides a potential therapy for neurodegenerative diseases. Antiapoptotic pathways and survival signal activation were increased in spinal cords of TTC-treated mice. These results also indicate that the delivery of this gene by means of intramuscular injection of naked-DNA is successful, plays a significant role in neuroprotection and delays disease progression.

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THEME 2 *IN VIVO* EXPERIMENTAL MODELS

P36 NEUROPROTECTIVE PROPERTIES OF EGF AND GHRP-6 IN AN ANIMAL MODEL OF AXONAL STRANGULATION

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Keywords: 1,2 diacetylethylbenzene, epidermal growth factor, growth hormone releasing factor six

Background: Neurotoxic effects of 1,2 diacetylethylbenzene (1,2 DAB) depend on both induction of protein aggregates between neurofilaments and microtubules, affecting their normal association cytoarchitecture and on generation of reactive oxygen species, which has been considered as a pathophysiological feature of ALS (1,2). Epidermal Growth Factor (EGF) and Growth Hormone Releasing Peptide-6 (GHRP-6) have been endowed with a variety of pleiotropic and survival mechanisms, which turn different cell lineages into more tolerant ones before a broad spectrum of insults (3,4).

Objectives: To evaluate the separate and combined effects of EGF and GHRP-6 in an experimental animal model of ALS, the 1,2 DAB induced-axonopathy.

Methods: C57BL/6 mice were injected with a daily dose of 30 mg/kg of 1,2 DAB for up to 6 weeks. Subsequently, four groups of 15 animals each received daily injections of EGF (200 μl/kg), GHRP-6 (660 μl/kg), EGF with GHRP-6, and saline, correspondingly, until day 65. The evaluations were conducted by means of behavioral tests (trapeze, tail suspension, gait pattern, and open field) and by recording the complex muscle action potential (CMAP) in three different hind limb segments: proximal (S1), medial (S2) and distal (S3).

Results: Intraperitoneal administration of 1,2 DAB produced: significant reduction in body weight, muscle strength, extensor reflex and spontaneous activity, as well as changes in gait pattern parameters. In parallel, 1,2 DAB produced a significant prolongation and decrease in CMAP onset latency, amplitude as well as a decrease in integrated complex action potential index (ICMAPI). EGF and GHRP-6 separately were ineffective in attenuating the toxic clinical effect in 1,2 DAB treated animals except for the gait pattern. However, the combined treatment produced significant improvements in most behavioral and electrophysiological parameters, particularly in segment S1.

Conclusions: Axonal pathology induced by 1,2 DAB seems to be a reliable etiopathogenic animal model for ALS, which has also been related with toxic etiology. The concerted actions of EGF and GHRP6 seem to create the appropriate homeostatic background to protect neural cells from death and to stimulate neural tissue regeneration. Combined therapy with EGF and GHRP-6 might be of therapeutic interest in ALS.

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P37 EFFECT OF THE NOGO RECEPTOR ANTAGONIST NEP1-40 ON THE TIBIALIS ANTERIOR MUSCLE OF THE SOD1 G93A MOUSE MODEL OF MND/ALS

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Keywords: SOD1 mice, plasticity, NOGO

Background: Compensatory synaptic re-organisation occurs in ALS patients and murine models (1), but may be restricted by release of neurite outgrowth inhibitors like NOGO. It has previously been shown that in the tibialis anterior muscle of $SOD1^{G93A}$ mice (2) preferential atrophy with increased NOGO expression occurs in superficial regions (composed of Type 2B fibres), but lesser atrophy and altered NOGO expression occurs in deeper regions comprising of Type 2A/I fibres.

Objectives: To investigate the effect of NEP1-40, a competitive antagonist of the NOGO receptor on muscle morphology.

Methods: Male $SOD1^{G93A}$ mice were divided into 3 groups of 10. Control transgenic (Tg) $SOD1^{G93A}$ mice and age-matched wild-type littermates (Wt) were injected intraperitoneally with sterile PBS-17% DMSO, Tg $SOD1^{G93A}$ mice received NEP1-40 (Alpha Diagnostic; 4 mg/kg/day(d)). Dosage commenced at 13 weeks, onset of NOGO response (2). A set of 3 mice (2 control, 1 NEP treated) were culled when a control Tg mouse became symptomatic (119–125d). Three sets were processed for high resolution histology; 3 sets were processed for phosphorylated GAP43 immunocytochemistry. Remaining sets provided tissue homogenates for Western blotting. Focus here is on the tibialis anterior muscle.

Results: 1) All control Tg mice were symptomatic, 9/10 NEP-treated mice were asymptomatic. 2). Relative to Wt controls, fibres in Tg controls reduced by 40–50% of normal area in superficial regions of the TA ($p < 0.001$), but 20–30% in deeper regions ($P = 0.005$). Relative to Tg controls, fibres in NEP1-40 treated Tg mice were up to 17% larger in superficial regions ($Tg:Tg^{NEP}$ difference $P < 0.02$), and 20% in deeper regions ($P < 0.02$). 3). Compared to the absence of myelinated axon damage in intramuscular nerves to Wt mice, about 90% were abnormal in Tg controls, but only 30% in NEP treated mice. 4). Western blots of phosphorylated GAP43 in TA muscles produced bands at 47 kDa. Relative to Wt mice, expression in Tg controls, measured as integrated

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optical density, increased by 30% and further increased by 23% in NEP treated mice. 5) Immunocytochemistry revealed a low incidence of phosphorylated GAP43 immunoreactive profiles throughout the TA in Wt mice (measured as number of profiles/unit area).Incidence increased up to 700% in Tg controls and 600% with NEP treatment.

Conclusions: A blockade of NOGO receptor NgR by the competitive antagonist NEP1-40, at a dose level shown to promote axon growth in spinal cord injury (3), improves hindlimb muscle fibre size, reduces degeneration of intramuscular axons and supports expression of the phosphorylated form of GAP43. Continuing studies examine the effect of higher dosage and the response in the spinal cord.

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P38 OVEREXPRESSION OF GLIAL METALLOTHIONEIN SLOWS DISEASE PROGRESSION IN A MOUSE MODEL OF MUTANT SOD1-LINKED FAMILIAL ALS

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Keywords: metallothionein, non-neuronal cell, transgenic mouse

Background: Mutations in the copper/zinc superoxide dismutase (SOD1) gene have been found in familial amyotrophic lateral sclerosis (ALS). Non-neuronal cells, including glial cells, shape motor neuron survival in ALS and are a potential target to prevent motor neuron degeneration. Metallothionein-I (Mt-I), a metal binding protein, is primarily located within glial cells. When neurons are injured, Mt-I is released by glial cells toward the injured neurons where it acts to promote neuronal survival and regeneration. In fact, Mt-I lacking-mutant SOD1 mice have a shorter survival and accelerated motor neuron death. Despite the fact that Mt-I plays a critical role in protecting motor neurons, it is not known whether overexpression of Mt-I ameliorates mutant SOD1 toxicities.

Objectives: To elucidate the effects of Mt-I overexpression on the disease.

Methods: Overexpressing mice with a high copy number of human SOD1 with G93A mutation ($SOD1^{G93A}$) and overexpressing mice with mouse Mt-I (Mt-I*) were purchased from Jackson Laboratories. To minimize the differences caused by strain background, a two step breeding strategy was performed. Heterozygous male $SOD1^{G93A}$ were crossed with homozygous female Mt-I*. Heterozygous male Mt-I* with $SOD1^{G93A}$ were then intercrossed with heterozygous female Mt-I* to obtain the homozygous Mt-I* with $SOD1^{G93A}$ (Mt-I*/$SOD1^{G93A}$) mice.
The clinical onset of the disease was evaluated by examining mice to identify shaking of the limbs when suspended in the air by the tail. The end-point was defined as the inability of a mouse to right itself within 30 seconds after being pushed onto its side. The progression of the disease, duration of disease or survival after onset, was calculated as the period between the onset and the endpoint of the disease.
For analysis of the survival of the motor neurons, lumbar sections of the spinal cord were immunostained using anti-NeuN antibody (1:1000, Chemicom). A total of 10 lumbar sections were analyzed per mouse. The number of NeuN-positive neurons (>25 mm) in the ventral horn was counted.

Results: Mt-I*/$SOD1^{G93A}$ mice showed a significant delay in the disease onset by 7.2%. Overexpression of Mt-I dramatically prolonged the survival of the mice by 18%. Of note, overexpression of Mt-I significantly slowed the progression of the disease by 55%. At the clinical end-point, $SOD1^{G93A}$ mice had only 50% surviving motor neurons, whereas overexpression of Mt-I protected a significant proportion of motor neurons, representing approximately 68% of the total number of motor neurons, from death.

Conclusions: Mt-I might be a potential therapeutic target for familial ALS patients with SOD1 mutations.

P39 LIF PROMOTES THE EXPRESSION OF SYNAPTOPHYSIN ON MOTOR NEURONS IN THE SOD1 G93A G1H TRANSGENIC MOUSE MODEL OF FALS

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Background: Amyotrophic lateral sclerosis (ALS) is a common form of motor neuron disease (MND) that involves both upper and lower motor neurons. The $SOD1^{G93A}$ transgenic mouse is a widely used animal model of human ALS. To date the mechanisms of ALS are still unclear.

Objectives: To evaluate whether LIF can up-regulate the expression of synaptophysin on the motor neurons in the anterior horn of spinal cord and sensorimotor cortex in a transgenic mouse model of ALS.

Methods: Motor neurons from both LIF and Saline treated groups were labelled using Flurogold (FG) and synaptophysin protein was immunostained. The number and density of synaptophysin were estimated using the confocal counting system.

Results: The LIF groups showed a statistically significant improvement in the number and density of synaptophysin as compared with saline treated groups.

Discussion: The data from this study provides some insights into the changes in synaptic boutons on motor neurons that degenerate as ALS progresses. The loss of motor neurons and the decrease of synaptophysin in the remaining motor neurons may contribute significantly to the progression of ALS and the increase of synaptophysin in the remaining motor neurons of mice treated with LIF could lead to functional motor recovery.

Conclusions: LIF may be a useful method in ameliorating the motor functional behaviour for ALS in the future; its neuroprotection mechanism may contribute to the upregulation of the expression of synaptophysin.

P40 ENDOTHELIAL PROLIFERATION IN THE SPINAL CORD MICROVASCULATURE OF ALS TRANSGENIC RATS

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Keywords: SOD1, regeneration, angiogenesis

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by adult-onset selective motor neuron loss. Approximately 2% of all ALS cases are linked to mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Recently, disruption of the blood-spinal cord barrier has been reported as an early event in transgenic mice with ALS-linked mutant SOD1 genes, suggesting an important role of microvascular damage in the motor neuron pathology.

Objectives: To clarify the role of microvascular damage in neurodegeneration, we examined a possible reconstruction of microvasculature in the spinal cord of a transgenic rat model of ALS.

Methods: Angiogenesis was examined in lumbar spinal cord of His46Arg mutant SOD1 transgenic (Tg) rats at presymptomatic, early symptomatic and late symptomatic stages with their age-matched non-transgenic (non-Tg) littermates. Continuous administration of a thymidine analogue bromodeoxyuridine (BrdU) for 7 days labelled newborn cells *in vivo*. A multiple immunohistochemistry assay employing cell-selective markers in the lumbar spinal cord cryosections was then performed. In addition, we quantified the immunofluorescence under confocal laser-scanning microscopy to determine the number of microvascular newborn endothelial cells and neural progenitors using computer-based software with digitally-captured microscopic images.

Results: In contrast to non-Tg rats, the Tg rats showed a significant increase of microvascular endothelial regeneration especially at the site of neurodegeneration in the ventral spinal cord. The microvascular reconstruction peaked at the early symptomatic stage, followed by a decrease at the late symptomatic stage in Tg rats. Neuropathology such as loss of ventral horn neurons, gliogenesis, ubiquitinated protein aggregates and phosphorylated neurofilamentous accumulation progressed consistently as reported previously.

Discussion and Conclusions: An intrinsic regenerative response against the microvascular damage has been identified in an ALS rat model. Therefore, microvasculature may be considered as a potential therapeutic target for neurodegenerative diseases such as ALS.

P41 IMPACT OF THE NEUROTROPHIC FACTOR FGF-2 ON THE NEURODEGENERATIVE DISORDER ALS: EVIDENCE FROM MUTANT MICE

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Background: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by selective motoneuron loss in the brain and spinal cord. Mutations in the superoxide dismutase (SOD) 1 gene are detected in 10–20% of familial ALS patients. The ALS-mouse model overexpressing a mutant human SOD1 (G93A) gene is similar to the human ALS disease. The cause for the selective death of motor neurons is still unclear, but many different pathomechanisms are discussed including loss of neurotrophic factors. One of the discussed neurotrophic factors that could play a prominent role in the motor system is basic fibroblast growth factor (FGF-2).

Objectives: To evaluate the physiological role of FGF-2 in the ALS scenario, we established double mouse mutants transgenic for the human SOD1 mutation and lacking the endogenous FGF-2 gene.

Methods: By using PCR, double mouse mutants were detected. Approximately 60% of our offspring survived up to genotyping and only 35% of those had a double mutation instead of the expected 50%. Double mutants that are heterozygous for FGF-2 have been evaluated so far. Evaluation parameters include lifespan and survival of spinal motoneurons compared to SOD1 mutant mice. Motor performance of different mutants was tested by rotarod.

Results: FGF-2 deficient mutant SOD1 mice demonstrate a later onset and also perform better during the rotarod-test in comparison to mutant SOD1 mice. Also, the life expectancy of the double mouse mutants was enhanced. Up to now there has been no difference in the number of lumbar motoneurons in the end stage of each group. The investigation of motor neuron numbers is ongoing.

Discussion and Conclusions: The unexpected results could suggest that FGF-2 is involved in cell death and oxidative stress pathways. On the other hand, the FGF-2 deficit could also be balanced via an upregulation of other neurotrophic factors. Further exploration of these hypotheses will be performed. Due to the neurodegeneration in ALS being possibly caused by a dying back mechanism, the neuromuscular end plate of the hind extremity muscle (M. gastrocnemius) and also the purely motor cranial nerve (N. hypoglossus) will be examined. Motoneuron numbers will be determined to a consistent earlier time point. These results could be important to understand the underlying pathomechanisms of ALS and help to develop new therapeutic strategies for patients with motoneuron disorders.

P42 TRANSPLANTATION INTO CISTERNA MAGNA USING HUMAN MESENCHYMAL STEM CELLS OBTAINED FROM A PATIENT WITH AMYOTROPHIC LATERAL SCLEROSIS: PROLONGED SURVIVAL AND DELAYED DISEASE PROGRESSION

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Keywords: mesenchymal stem cells, cisterna magna

Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative condition characterized by motor neuron loss. Although the underlying cause of the disease remains unclear, a variety of pathogenic mechanisms have been proposed. However, despite promising preclinical studies showing the modification of the disease progression,

most trials have failed to demonstrate any significant improvement. Therefore, stem cells have been proposed as an alternative therapy for ALS.

Objectives: To evaluate the dose-dependent effect of human bone marrow mesenchymal stem cells (hMSCs) obtained from an ALS patient on G93A mice using an intrathecal route of administration.

Methods: Different doses (2×10^4, 2×10^5, 1×10^6) of hMSCs obtained from an ALS patient were transplanted into cisterna magna of asymptomatic G93A mice. Clinical observations were performed including symptom onset and survival time and body weight. Locomotor performance was measured by performing a rotarod test on these mice. For motor neuron counting, nissl staining was processed in the lumbar spinal cord region sections of euthanized mice at 109 days. Transplanted cells were detected by immuno-fluorescence staining at the end stage.

Results: A cell dose of 1×10^6 significantly increased life span and improved the motor performance. Its average motor neuron count significantly increased compared to other groups. Most of the injected hMSCs were observed in subarchnoidal space, especially the fourth ventricle and a few in spinal cords.

Conclusions: These data suggested that intrathecal injection might be a practical potential route for stem cells therapy in ALS if the maximum number of cells is used. Investigation into the neuroprotective mechanisms of hMSCs is essential to further research.

P43 EXPOSURE TO STERYL GLUCOSIDES *IN VIVO* CONTRIBUTES TO DISEASE PROGRESSION IN THE MSODG37R LINE 29 MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: steryl glucosides, cycad, neurodegeneration

Background: Missense mutations in the gene encoding superoxide dismutase 1 (SOD1) are reported to be one cause of adult-onset familial amyotrophic lateral sclerosis (fALS). However, fALS makes up only 10% of all ALS cases, while the remainder of ALS cases are sporadic with unknown causes, thought to be of environmental origin. It has previously been shown that an ALS-Parkinsonism dementia complex (PDC) phenotype can be demonstrated by feeding mice cycad seed flour as well as several various neurotoxic steryl glucosides isolated from this flour. The influence of dietary exposure of β-sitosterol β-D-glucoside (BSSG) has been studied in a transgenic mouse line (line 29) overexpressing the human $SOD1^{G37R}$ mutant. BSSG is the largest fraction of steryl glucoside in cycad seeds.

Objectives: To test the effect of dietary steryl glucosides on the disease onset, duration, and neurodegeneration of adult onset ALS using the $mSOD1^{G37R}$ mouse model.

Methods: Experimental cohorts of mice were given 42 mg BSSG/kg body weight prepared daily as a pellet of 1.5 g and given at a fixed time of day. Control animals received daily allowances of normal mouse chow pellets to ensure an equivalent weight of food. Animals were weighed weekly to assess for changes in metabolism. Motor function and coordination were assessed weekly by rotarod and wire hang (grip strength) performances. Indications of motor neuron dysfunction were measured with hind limb clasping reflex scores. Parameters of gait abnormalities and open field roaming activity were monitored at 5, 7 and 9 weeks of age using DigiGait and EthoVision, respectively. At the end state of disease, marked by the onset of hind limb paralysis, animals were perfused and processed for histological assessment of motor neuron survival and characteristics of neurodegeneration in the motor system from the upper motor neurons to neuromuscular junction.

Results: These results demonstrate that exposure to multiple stressors, genetic and environmental, lead to a more pronounced phenotype than either stressor alone. These include a more rapid and steeper decline in leg extension scores and grip strength, increased astrocyte accumulation around ventral motor neurons, and fewer surviving motor neurons in the spinal cord. Histology of brain and muscle is currently in progress.

Discussion: Although the mechanism of cycad toxin-induced neurodegeneration remains uncertain, the link between our current data and the epidemiological studies of ALS-PDC seems well founded. These results showed that dietary exposure to steryl glucosides alone was sufficient to produce a disease phenotype and when implemented in conjunction with a genetic predisposition to ALS was sufficient to produce a remarkably more severe disease phenotype.

Conclusions: These data suggest that environmental influences contribute to disease progression in ALS and point to a dynamic interplay of genes and environment in the etiology of ALS and perhaps other neurodegenerative disorders.

P44 FETAL AND SECONDARY ADULT TREATMENT WITH STERYL GLUCOSIDES PRODUCES DIFFERENTIAL BEHAVIORAL AND NEUROPATHOLOGICAL OUTCOMES IN MALE AND FEMALE MICE

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Background: Amyotrophic lateral sclerosis-parkinsonism dementia (ALS-PDC) is a neurodegenerative disease first characterized among the Chamorros of Guam. Symptoms present as a spectrum, but usually express a dominant phenotype of ALS or PDC. Neuroepidemiological evidence has linked consumption of the seeds of the cycad Cycas micronesica to ALS-PDC. Two water insoluble steryl glucosides (stigmasterol β-D-glucoside (SG), and β-sitosterol β-D-glucoside (BSSG)) identified in washed cycad have previously been demonstrated to have neurotoxic effects both *in vitro* and *in vivo*. Epidemilogical studies showed that exposure to unknown environmental factor(s) prior to adulthood was predictive of future disease expression. To study this, the impact of a prenatal and secondary adult re-exposure

to a combination of BSSG and SG on disease onset and progression in male and female mice was investigated.

Objectives: To examine the interrelated hypotheses that toxin exposure during fetal development will (a) target particular neuronal populations, (b) impact those same neurons making a secondary exposure more severe and (c) express differentially in males and females.

Methods: Pregnant CD-1 mice were treated by diet with 0.6 mg BSSG and 0.4 mg SG on embryonic days 10–11 to target the differentiation of the nigro-striatal system; controls received normal diet. Pups were weighed weekly and monitored behaviorally starting at 5 weeks of age for the duration of the study. At 18 weeks, subsets of both litters were exposed to dietary steryl glucosides as before for 15 weeks before sacrifice. Regions of the CNS were examined by MRM and histological methods.

Results: Significant differences were found in the responses of both male and female mice to prenatal steryl glucoside exposure. Male mice showed significant weight increases along with motor behavioral deficits. Female mice showed marked hyperactivity if they received the toxins as adults. Male mice showed significantly decreased tyrosine hydroxylase (TH) labeling in the nigro-striatal pathway, increased apoptosis and gliosis, as well as widespread lipid accumulation in lumbar spinal cord. Diffusion tensor imaging (DTI) data in the lumbar spinal cord also showed toxin - induced reductions in fiber density as well as an increased diffusion coefficient (ADC) indicative of axonal disruption. Female mice did not show significant changes in the CNS.

Discussion and Conclusions: Prenatal exposure to steryl glucoside neurotoxins has significant pathological impacts on the CNS of male mice that are worsened by adult exposure. Female mice appear to show recovery from the prenatal insult, unless re-exposed as adults.
Developmental exposure to cycad-derived neurotoxins elicits a dominant pathological phenotype based on the period of exposure. The fetal insult can be exacerbated by a secondary exposure depending on animal sex.

P45 NEURONAL OVEREXPRESSION OF CHROMOGRANIN A ACCELERATES MOTOR NEURON DEGENERATION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: chromogranins, mutant SOD1, degeneration

Chromogranins, which are abundant proteins in motor neurons and interneurons, promote selective secretion of misfolded SOD1 mutants. Extracellular mutant SOD1 can induce microgliosis and motor neuron death, suggesting that chromogranin-mediated secretion of mutant SOD1 proteins could be a pathogenic mechanism in ALS.
Toward a better understanding of this mechanism, a transgenic mouse line expressing mouse CgA protein fused with hemagglutinin (HA) under the control of human Thy1 promoter was generated. Double transgenic mice overexpressing G37R SOD1 together with CgA were generated through breeding of G37R SOD1 mice with mice hemizygous for CgA transgene. Motor neuron pathology was analyzed at presymptomatic, early symptomatic and late symptomatic stages of the disease.
These results demonstrate that neuronal overexpression of CgA is associated with an increase in motor neuron degeneration at the levels of neuromuscular junctions, ventral roots (L5) and spinal cord. In addition, immunoprecipitation using monoclonal antibodies specific to misfolded mutant SOD1 exhibited a higher level of misfolded SOD1 species in double transgenic mice compared to G37R mice, suggesting that excess of CgA may promote stabilization of pathogenic SOD1 species.
Moreover, G37R mice lacking CgA have been generated by crossing a CgA knock out mouse with G37R SOD1 mouse. Quantitative analysis of motor neuron loss showed a decreased level of degeneration in CgAKO G37R mice at late symptomatic stage, as indicated by a higher number of motor axons in the ventral root (L5).
These results support a role for chromogranins in ALS pathogenesis caused by SOD1 mutants.

P46 THE G93A SOD1 GENE MUTATION MODIFIES THE ACUTE RESPONSE TO MILD SPINAL CORD COMPRESSION: FUNCTIONAL AND MOLECULAR OBSERVATIONS

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Keywords: spinal cord injury, gene expression

Background: The presence of a SOD1 gene mutation or of other genetic liabilities to develop amyotrophic lateral sclerosis (ALS) may affect the molecular response to trauma in a pre-symptomatic stage.

Objectives: To compare the acute response to a mild compression spinal cord injury (SCI) of pre-symptomatic rats over-expressing the G93A SOD1 gene mutation with their wild type (WT) littermates.

Methods: Large-scale gene expression analysis of lumbar spinal cord samples from mutant and WT rats at different time-points within a 7-day post-injury period has been obtained using an Illumina Bead-array system, combined with an ontological analysis of the main gene expression changes specific to each genetic type. Within the same post-injury time-window, we have performed accurate loco-motor analysis to identify differences in functional recovery between the two groups of animals in study. The post-injury differential regulation of selected gene candidates has been confirmed by RT-PCR and by using immunohistochemistry.

Results: Gene expression analysis of injured spinal cord has identified genotype-specific patterns of differential regulation, including a 4-hour post-injury induction of pro-apoptotic genes involved in cytochrome-C release in mutant spinal cord. In WT spinal cord, a predominant pro-survival response after the 4 hours time point includes the activation of genes regulating cell cycle, angiogenesis, lipid transport, cell adhesion and the formation of the extra-cellular matrix. Retinoid signalling is activated predominantly in mutant spinal cord at 7 days from injury, when genes involved in ion current regulation and neurotransmitter transport become down-regulated only in wild type spinal cord. Intermediate neurofilaments become significantly down-regulated at 7 days from

injury in WT spinal cord, whilst maintaining a high level of expression in mutant tissue throughout the period of observation. Locomotor analysis demonstrates that wild type animals undergo a substantial functional recovery whereas mutant rats, though less functionally impaired at 4 hours, improve only marginally thereafter.

Discussion and Conclusions: Response to injury in mutant spinal cord shows distinctive molecular features in the activation of cell-death mechanisms and of retinoid signalling, in neurofilament homeostasis and in ion channel regulation. The identification of a post-injury molecular signature specific to mutant spinal cord is an important step towards the identification of those molecular processes that condition the susceptibility to neurodegeneration under conditions of mechanical stress.

P47 ATF-3 ACTIVATION AND THE DEVELOPMENT OF SPINAL CORD DEGENERATION IN A RAT MODEL OF ALS

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Keywords: ATF3, spinal cord

Background: The development of motor neuron loss in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder, is a poorly understood phenomenon. How and where the disease initiates and affects the spinal cord is also far from being clear.

Objectives: To use the activation of the ATF3 adaptive response to investigate early signs of cell distress in lumbar spinal cord and in dorsal and ventral roots from the G93A SOD1 rat model of ALS. We have also looked at those molecular responses that appear to be co-regulated with ATF3 spinal cord activation throughout the development of the disease.

Methods: Immunohistochemistry has been used to obtain a profile of the temporal and anatomic expression of ATF3 in lumbar spinal cord and afferent/efferent pathways from the G93A SOD1 animal model of ALS from the pre-symptomatic stage. We have utilised large scale gene expression analysis to characterise the molecular profile of lumbar spinal cord from the G93A SOD1 rat model of ALS at a pre-symptomatic stage, at onset and at end-stage disease. The ontological analysis of the gene expression datasets has shown those molecular pathways that are coregulated with the activation of ATF3.

Results: Large-size neurons along with non-peptidergic smaller neurons in dorsal root ganglia (DRG) showed ATF3 staining in an early pre-symptomatic stage, when no signs of motor cell involvement were detectable in spinal cord. These DRG changes were accompanied by ATF3 staining of Schwann cells in both rostral and ventral roots and, at an advanced stage of the disease, by intense ATF3 immunoreactivity in motor neurons in the spinal cord. The ontological analysis of the gene expression profile of lumbar spinal cord from the ALS rats shows ATF3 co-activation with gene categories heavily involved in the regulation of cell growth and protein metabolism. ATF3 over-expression seemed also to correspond to the activation of molecular pathways modulating TLR and innate immune responses, cell-cycle and Golgi and other organelle structural and functional integrity.

Discussion: Our study indicates that sensory pathways including muscle spindle afferents and ventral roots motor efferents are the first anatomic sites to become involved by the disease process and that multiple gene candidates which modulate growth share a similar temporal profile of tissue activation with ATF3 in spinal cord.

P48 GENE EXPRESSION PROFILE OF SPINAL ASTROCYTES IN THE G93A MOUSE MODEL

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Keywords: astrocytes, microarrays, metabolism

Background: Amyotrophic lateral sclerosis (ALS) is characterized by progressive death of upper and lower motor neurons (MN). Experiments on mutant SOD1 mice (1) have indicated that the development of the pathology requires interaction between MN and other cell types (2). Although the targeted expression of mutant SOD1 (mSOD1) in astrocytes (3) fails to provoke an ALS phenotype, the selective silencing of mSOD1 expression in astrocytes significantly slowed the disease progression in a mouse model of the disease (4). It is, therefore, clear that astrocytes play an important role in ALS, but in which way the expression of the mSOD1 *in-vivo* alters their properties has yet to be clarified.

Objectives: 1) To investigate the gene expression profile of astrocytes isolated from $SOD1^{G93A}$ mice and non-transgenic littermates at different stages of the disease, using laser capture microdissection (LCM) and microarray analysis 2) To identify pathways involved in the development of the neurodegenerative process 3) To investigate the cross-talk between astrocytes and MN isolated from mice at the same age(5) by comparing their transcription profile.

Methods: Three $SOD1^{G93A}$ mice and 3 non-transgenic littermates at 60 days were used. Approximately 1500 astrocytes have been isolated from ventral lumbar spinal cord sections immunostained with the astrocyte specific marker AldhL1l (6), using the Arcturus Pixcell 2 LCM Microscope. RNA was extracted using Picopure kit (Arcturus), amplified and labelled using the Affymetrix Two-cycle Amplification kit (Affymetrix). 10 μg cRNA was applied to the Affy MOE430 2.0 GeneChip and data analysis was performed using ArrayAssist System (Iobion).

Results: By comparing the expression profile of astrocytes from 60 days $SOD1^{G93A}$ mice and non-transgenic littermates a significant change in the expression of 1109 genes was found when applying a threshold of twofold or higher. 526 transcripts were downregulated, and 583 were upregulated. The genes have been categorised according to their molecular function, and include genes involved in the transcription process, apoptosis, inflammation and metabolism. $SOD1^{G93A}$ astrocytes show an increase in transcripts encoding for Ngf and several chemokines. One of the main features is an overall downregulation in genes involved in the carbohydrate metabolism opposed to upregulation in the main genes involved in fatty acid synthesis and activation. Several transcripts involved

in the WNT signalling cascade are also differentially expressed. This pathway is known to stimulate cell proliferation and cytoskeletal changes.

Discussion: Previous data obtained from the expression profile of MN isolated from $SOD1^{G93A}$ mice (5) suggest that carbohydrate metabolism dysregulation is one of the main features of the early stages of disease. We aim to investigate this dysregulation as a potential mechanism leading to motoneuronal damage.

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P49 ASTROGLIAL MONOCARBOXYLATE TRANSPORTER 1 (MCT1) IS NECESSARY FOR NEURON SURVIVAL IN THE RODENT SPINAL CORD

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Keywords: metabolism, lentivirus, lactate

Background: Several lines of evidence have suggested a role for astrocytes in the degeneration of motor neurons in amyotrophic lateral sclerosis (ALS). Astrocytes support the function of motor neurons through several mechanisms, including secretion of trophic factors and removal of glutamate from the synapse. In the brain, astrocytes also provide metabolic support for neurons in the form of the energy substrate, lactate. Astrocytes produce intracellular lactate through glycolysis and then export it through monocarboxylate transporters (MCT1 and MCT4) to the extracellular space where it can be imported into neurons via MCT2 to serve as an alternate source of energy.

Objectives: To investigate the hypothesis that the astroglial supply of lactate to motor neurons in the spinal cord is critical for their function and that motor neuron death could result from abnormal or reduced astroglial MCTs given that spinal cord motor neurons are metabolically active cells with a high demand for energy.

Methods: To determine whether the loss of the most abundant astroglial MCT, MCT1, is sufficient to produce spinal cord motor neuron loss, we pharmacologically inhibited MCT1 and down-regulated spinal MCT1 expression in both *in vitro* and *in vivo* model systems. Pharmacologic inhibition was through a recently published, specific inhibitor to MCT1. Down-regulation was accomplished *in vitro* by anti-sense oligonucleotides and *in vivo* by a lentiviral construct expressing shRNA specific for MCT1.

Results: In organotypic spinal cord cell cultures, we found that anti-sense oligonucleotides specific for MCT1 led to greater than a 50% decline in motor neurons, as quantified by counts of large neurofilament-positive neurons and a specific pharmacologic inhibitor to MCT1 led to cell death, particularly when combined with glucose deprivation, as quantified by propidium iodide fluorescence intensity. As would be predicted from the known function of MCT1 as an exporter of lactate, cell death secondary to the pharmacologic inhibitor was attenuated by providing exogenous lactate to the culture media. *In vivo*, we found that cervical spinal cord injections of a lentiviral construct expressing MCT1 shRNA, but not an identical lentiviral construct expressing only GFP, produced approximately 40% loss of motor neurons.

Discussion: These *in vitro* and *in vivo* studies provide strong evidence for a critical role of astroglial MCTs in the survival of motor neurons. Given the attenuation of cell death by exogenous lactate, the toxicity from reduced MCT1 activity appears to be due to reduced availability of lactate to motor neurons.

Conclusions: Given the recent findings of reduced MCT1 in spinal cords from ALS patients and a mouse model of ALS, these results provide fundamental evidence that reduced MCT1 is contributing to motor neuron degeneration in ALS and suggest novel targets for treating ALS patients.

P50 A ROLE FOR THE CATION CHLORIDE COTRANSPORTER, KCC2, DURING PATHOGENESIS IN THE SOD1-G93A MOUSE MODEL OF ALS

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Keywords: KCC2, excitotoxicity, hypoglossal nucleus

Background: In amyotrophic lateral sclerosis (ALS) some somatomotor neurons degenerate, while others survive. Since excitotoxicity is considered to play a causal role in the pathogenesis of ALS we speculated that a switch in post-synaptic responses to GABA- and glycine-signalling from inhibitory to excitatory may contribute to selective over-excitation and motor neuron death.

Objectives: To investigate the expression patterns of two members of the SLC12 family of cation chloride cotransporters, the Na-K-2Cl co-transporter 1 (NKCC1) and the K-2Cl co-transporter 2 (KCC2), proteins. These are crucial for inhibitory neurotransmission in post-synaptic neurons, during disease progression in the lumbar spinal cord, the oculomotor and the hypoglossal nucleus in the SOD1-G93A mouse model of ALS.

Methods: RT-PCR and *in situ* hybridisation histochemistry were used to assess NKCC1 and KCC2 transcript levels from postnatal day (P) 10 until P120. Immunohistochemistry was performed to detect KCC2 protein in tissue sections and a tongue motility paradigm was utilized to evaluate the degeneration status of the hypoglossal nucleus.

Results: NKCC1 transcipts were not present in any motoneuron population studied at any postnatal timepoint in control mice and during disease progression in SOD1-G93A mice. In contrast, KCC2 mRNA was present in all motoneuron populations analysed. At P110, KCC2 expression levels were found to be decreased by 20% in spinal cord motoneurons and by 17% in the vulnerable hypoglossal nucleus of SOD1-G93A mice, but not in the resistant oculomotor nucleus. Reductions in KCC2 immunoreactivity were observable beginning with P80 in the hypoglossal nucleus of SOD1-G93A mice. They first appeared in its ventral part, i.e. the genioglossal sub-nucleus and then spread over the entire nucleus until P120. In addition, KCC2 immunoreactivity was no longer confined to the cell membrane, as seen in control mice, but was found more dispersed

in the cytoplasm. Changes in KCC2 expression and localisation preceded tongue motility deficits which started at P110.

Discussion and Conclusions: While a role of NKCC1 for motor neuron pathology in this ALS model is unlikely, reductions in KCC2 expression levels and changes in its intracellular distribution pattern may contribute to excitotoxicity by reducing and/or switching inhibitory synaptic input to excitatory.

P51 PERSISTENT SODIUM CURRENTS ARE NOT ALTERED AT DISEASE ENDSTAGE IN VULNERABLE BRAINSTEM MOTONEURONS OF THE TRANSGENIC SOD1G93A ALS MOUSE MODEL

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Keywords: patch clamp, persistent sodium channel, excitability

Background: The mechanisms of selective motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS) are still unresolved. Electrophysiological studies on transgenic (tg) SOD1G93A ALS mice identified increased TTX-sensitive persistent sodium currents in presymptomatic cultured embryonic or early postnatal ($\leq$10 days) motoneurons, which were accompanied by increased cellular excitability (1,2). Accordingly, enhanced persistent sodium channel activity was proposed as a candidate mechanism for an overexcitability-mediated MN death in ALS.

Objectives: To identify whether changes of persistent sodium channel activity were still present in the relevant time window between 3 and 4 months of age, when vulnerable MN populations in SOD1G93A mice degenerate and thus lead to ALS-like motor symptoms and finally death.

Methods: To address this issue, we developed a brainstem slice preparation to perform whole-cell patch clamp recordings on adult vulnerable hypoglossal MNs during disease onset, progression and up to disease endstage.

Results: Investigating electrophysiological parameters of tg MNs in late disease stages (4 months) and age-matched wildtype controls (wt), we observed no differences in the properties of persistent TTX-sensitive sodium currents (current density: wt: -4.59 ± 0.34 pA/pF, n = 15; tg: -5.05 ± 0.53 pA/pF, n = 16; P = 0.48; voltage dependence of activation: V_{50}: wt: -37.48 ± 1.19 mV, n = 15 tg: -39.05 ± 1.05 mV, n = 16; P = 0.38, slope: wt: 5.85 ± 0.58 mV, n = 15; tg: 5.18 ± 0.40 mV, n = 16; P = 0.34). There was also no significant difference in the frequency-current (f-I) slope (wt: 55.2 ± 6.5 Hz/nA, n = 33; tg: 59.1 ± 4.2 Hz/nA, n = 30; P = 0.33), mean firing rate (e.g. at 600 pA-injected current: wt: 39.49 ± 2.93 Hz, n = 33; tg: 38.89 ± 2.61 Hz, n = 30; P = 0.89) and current threshold for the initial spike (wt: 279.0 ± 21.5 pA; n = 33; tg: 268.5 ± 27.6 pA, n = 30; P = 0.76) between tg and control MNs in this age group.

Discussion and Conclusion: While the persistent sodium current densities of adult wt and tg MNs were similar to those reported for young wt mice (2), other electrophysiological properties dramatically changed with the maturation of MNs (e.g. f-I gain was reduced 4-fold in adult compared to postnatal MNs).
These data indicate that changes in persistent sodium channels and related parameters of cellular excitability identified in MNs from embryonic and young postnatal presymptomatic ALS mice are not present during the active phase of degeneration, which only starts after 3 months of age. Thus, it might be unlikely that persistent sodium channels play an active degeneration-related role in the SOD1G93A mouse model of ALS.

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P52 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF DISEASE PROGRESSION IN SOD1G93A RATS

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Keywords: transgenic SOD1 rat, electrophysiology, segmental progression

Background: Recent data suggests that motor neuron degeneration in Amyotrophic Lateral Sclerosis/Motor Neuron Disease (ALS/MND) shows segmental differences along the neuraxis at onset. Understanding the onset, spatial and timely progression of the disease in ALS/MND animal models may help develop better outcome measures for testing new therapies and identify markers of disease progression. Transgenic $hSOD1^{G93A}$ rats like humans develop age dependent focal onset disease and are useful for preclinical testing.

Objectives: To characterize disease onset and segmental progression in pre- and symptomatic stages of the $hSOD1^{G93A}$ rat by electrophysiological means.

Methods: This study analyzed 16 female and 18 male SOD1 and control rats by serial electromyography (EMG) and nerve conduction studies (NCS) at presymptomatic (55 to 60 days), early disease onset (95 to 105 days) and late disease stages (150 days) in both upper and lower limbs. Acute denervation was monitored for each group in the tongue and in the distal leg muscles by needle examination (NE). Animals were also observed clinically for changes in body weight, limb atrophy, early gait difficulty and overall motor activity.

Results: Early segmental differences were found by EMG/NCS of male (m)SOD1 rats at disease onset (100 days of age) documented by reduced front paw distal compound muscle action potential (CMAP) amplitudes compared to controls (3.3 vs. 5.5 ms) and age matched female (f)SOD1 rats. Footpad mean CMAP amplitudes (5.4 and 5 mV respectively) and recording at the calf with stimulation at the sciatic notch showed no differences between (m)SOD1 and wild type littermates at 100 days. NE confirmed ongoing denervation in the tongue in males with front paw CMAP reduction. On the contrary, female SOD1 rats showed a decline in mean CMAP amplitude at footpads compared to control littermates. SOD1 males had reduced body

weight compared to controls at 100 days of age while female weight remained stable longer.

Discussion: Upper limb CMAP amplitude reduction was associated with earlier clinical disease onset in mSOD1 rats only. Female SOD1 rats showed early CMAP amplitude reduction in the most distal leg first. These electrodiagnostic findings correlated with our clinical observations that in fSOD1 rats, lower footpad CMAP amplitudes frequently indicate hind limb onset, while mSOD1 rats with upper limb onset display occasional respiratory distress during anesthesia. These results provide a meaningful clinical marker for early disease progression.

Conclusions: EMG/NCS study may reveal early focal disease onset in the hSODG93A rat model of ALS/MND. Further studies are needed to correlate these focal findings with additional clinical, histological, and molecular measures to be able to utilize it in preclinical testing.

P53 SCIATIC NERVES OF TRANSGENIC SOD1 ALS MICE SECRETE CYTOKINES AND CHEMOKINES THAT AFFECT MACROPHAGE RECRUITMENT AND ACTIVATION

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Keywords: SOD1 G93A, PNS, macrophage

Amyotrophic Lateral Sclerosis (ALS) is a fatal, progressive neurodegenerative disease that affects the upper and lower motor neurons. A hallmark of ALS pathology in the peripheral nerves is neuromuscular junction orphaning and demyelination, suggesting a chronic injury state. The transgenic mouse hSOD1G93A (B6SJL-Tg(SOD1-G93A)1Gur), engineered to over-express the mutant human SOD1, recapitulates the disease course of human ALS.

In an effort to identify potential therapeutic targets, whole genome transcriptional profiling studies of hSOD1G93A skeletal muscle have been conducted. Here, the identification of transcriptional interaction networks underlying an activated macrophage signature in hindlimb muscle at the early presymptomatic phase (day 50) and throughout the disease course are detailed.

Unexpectedly, immunohistochemical studies demonstrated that CD68+ macrophages localize to distal axons and large caliber nerves rather than fibrotic muscle fibers. Furthermore, peripheral nerves accumulate activated macrophage, but not neutrophils, with a pronounced increase of cell numbers upon onset of clinical symptoms. Macrophage accumulation occurred early with a 6 fold greater number of foamy, CD68+ cells at day 60 relative to the non-transgenic controls and accelerated with disease progression. To extend on the *in vivo* observations, the chemokine and cytokine profile of the SOD1 G93A (B6SJL-Tg(SOD1-G93A)1Gur) peripheral nerve was investigated. Macrophages were recruited using a primary co-culture model originally developed to study macrophage and sciatic nerve interactions (1). In this co-culture model stimulated inflammatory macrophages phagocytose myelin when cultured with injured sciatic nerve thus providing a controlled system to study secreted soluble factors that affect macrophage activation. Stimulated peritoneal macrophages were collected and co-cultured with either SOD1 G93A injured sciatic nerve or wild-type sciatic nerve. SOD1 G93A and wild-type sciatic nerve supplemented with IFNγ were also cultured alone.

This study tested the hypothesis that the diseased SOD1 G93A sciatic nerve is in a chronic inflammatory state and thus would present a unique cytokine/chemokine profile relative to age matched, non-transgenic controls.

Sciatic nerves and peritoneal macrophages were prepared from day 90 SOD1 G93A mice and age matched non-transgenic littermates. Following co-culture, conditioned medium was collected for analysis by ELISA on days 2, 6, 12 and 14. As a primary screen for secreted factors, Quansys multiplex ELISA technology was utilized. Using this assay system conditioned medium was simultaneously screened for a panel of 16 different cytokines and chemokines. Markedly increased levels (4.5 fold increase at all time points) of IL-6 were found relative to non-transgenic control co-cultures. Additionally, IFNγ, MCP-1 and RANTES were present in both transgenic and control co-cultures at similar levels. Cultures of macrophages alone yielded no significant increases of any of the 16 cytokines or chemokines. Based on the co-culture results we undertook an immunohistochemical survey of SOD1 G93A sciatic nerve during the disease course. Strikingly, extensive staining of Schwann cells was found, but not CD68+ macrophage for IL-6 and MCP-1 from day 50 on, well before the onset of clinical symptoms. Taken together these results provide evidence that nerves of the PNS in SOD1 G93A mice are in a chronic injury state and suggest that there are soluble factors secreted by Schwann cells that affect macrophage recruitment and activation.

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P54 ULTRASTRUCTURAL DIVERSITY OF INCLUSIONS FOUND IN LUMBAR SPINAL CORD OF TRANSGENIC MICE THAT EXPRESS HUMAN SOD1-G93A

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Keywords: transgenic mice, spinal cord, ultrastructural pathology

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that mainly affects motor neurons in cortex, brain stem and spinal cord. ALS occurs in both sporadic and familial forms, which are clinically and pathologically similar. About 5–10% cases of ALS are familial, 20% of which carry SOD1 mutations. SOD1-G93A transgenic mice show an ALS-like phenotype and are widely used in ALS pathogenic and therapeutic researches. General pathological changes revealed in transgenic mice, include motor neuron loss, astrogliosis and vacuolar degeneration. Aberrant accumulation of neurofilaments and inclusions are usually observed. However, the diversity of ultrastructural pathology, especially that of inclusions has not been reported.

Objectives: To develop an intensive examination of the ultrastructural changes of lumbar spinal cord in SOD1-G93A transgenic mice at different stages of disease, especially the diversity of inclusions.

Methods: SOD1-G93A transgenic mice were purchased from Jackson Laboratory (Bar Harbor, ME). The lumbar spinal cord from 60 days old, onset and end stage SOD1-G93A transgenic

and age-matched control mice were used for light and transmission electron microscope examination.

Results: Besides motor neuron loss and reactive astrocytes, numerous eosinophilic inclusions were found in axons, especially in the cytoplasm of glial cells and infrequently in the perikaryon of motor neurons. The number of inclusions increased with the disease progression. We performed elaborative observation on ultrastructural changes of motor neurons and their axons, glial cells and ventral roots of lumbar spinal cord. The most noteworthy was the morphological diversity of inclusions which manifested differently according to the specified locations. Filament-rich inclusions were mainly observed in the perikaryon and proximal axons of motor neurons. Filaments and granular materials were found in the center and microtubules surrounding in the peripheral, or compacted filaments in the center and loosely interlaced filaments in the peripheral, or electron-dense granular materials in the core and filaments in the peripheral. Inclusions found in glial cells were mainly composed of electron-dense aggregates. Ramified tubule-formed inclusions were seen in the axons of large myelinated nerve fibers in the lumbar ventral roots. In addition, aggregated mitochondria and abnormal membranous structures which might derive from the expansion and degeneration of mitochondria were observed occupying the whole axonal transverse plane.

Discussion: This study provides detailed ultrastructural changes of motor neurons and their axons, glial cells and ventral roots of SOD1-G93A mice. Special emphasis was put on the multiple characteristics of inclusions. The results provide further information to the pathological changes in SOD1-G93A mice. However, the exact components of the inclusions remain to be exploited.

Conclusions: Mutant SOD1 causes not only motor neuron pathology but also glial changes. The ultrastructural pathologic features appear to be diverse in different cells and different portions of the same cell type.

P55 DIFFERENT PROTEIN AGGREGATION IN TWO SOD1G93A MOUSE MODELS OF ALS INDICATES DIFFERENT STRAIN-SPECIFIC MOTOR NEURON DISEASE PHENOTYPES

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Keywords: SOD1G93A mice, genetic background, protein aggregation

Background: Amyotrophic Lateral Sclerosis (ALS) is an heterogenous disease with respect to its clinical, biochemical and genetic features. Such heterogeneity is likely due to different genes and environmental factors that influence the disease course and the response to treatment in sporadic and even familial ALS. This variability makes assessments of potential treatments difficult. SOD1G93A transgenic mice represent the most valuable model for studying the pathogenesis of motor neuron disease. Recently, it was observed that the phenotype of two genetically different strains of SOD1G93A transgenic mice (129Sv and C57Bl6J) differed remarkably in terms of speed of symptom progression and survival length, the 129Sv strain being more severely affected. Both strains carry identical human SOD1G93A transgene copies and express the same amount of human SOD1 mRNA and protein in the spinal cord.

Objectives: To identify molecular mechanisms that may be associated with the difference in severity (or protection) of motor neuron disease, the histopathological features and some biochemical markers of the pathology in the spinal cord of the two SOD1 mutant mice strains at different stages of the disease were compared.

Methods: Immunohistochemistry for Choline Acetyltransferase (ChAT) and Glial Fibrillary Acid Protein (GFAP) was used to label respectively motor neurons and astrocytes. Electron microscopy analyses were performed in lumbar spinal cord sections from the two mice strains at comparable presymptomatic, early symptomatic and clearly symptomatic stages. Moreover, the levels of P-p-38MAPK, detergent-insoluble proteins and proteasome subunits were investigated by immunoblot analyses on spinal cord homogenates.

Results: In presymptomatic 129SvG93A mice a marked swelling and vacuolization of myelinated axons was present in the ventral horn and at symptomatic stage several hyaline inclusions appeared around motor neurons. In C57G93A mice a massive vacuolization of motor neuron cell bodies was observed at presymptomatic stages, swelling and vacuolization of myelinated axons were present at symptomatic ages and hyaline inclusions were detectable only at the advanced stage of the disease. Immunoblot analyses showed significantly higher levels of detergent-insoluble human SOD1 in the spinal cord of 129SvG93A mice at the early symptomatic stage compared to the C57G93A at the same age. A decrease of 20S proteasome subunits was found earlier during disease progression in 129SvG93A compared to C57G93A mice.

Conclusion: The presence of numerous hyaline inclusions and the higher levels of detergent insoluble proteins in the spinal cord of symptomatic 129SvG93A mice with respect to the C57G93A mice at a comparable disease stage suggest that an impairment in protein degradation might be predictive of a rapid disease progression. This is probably related to an early decrease in the proteasome function. Other mechanisms that may contribute to the different phenotype are under investigation.

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P56 GENERATION AND CHARACTERIZATION OF CONGENIC LINES OF MUTANT SOD1 TRANSGENIC AND ALS2 KNOCKOUT MICE

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Keywords: mouse model, genetic background, lifespan

Background: Genetically engineered mice are among the central tools for disease studies not only on the molecular pathogenesis but also on the development of therapeutics. In fact, the creation of ALS animal models, namely transgenic (tg) mice expressing a disease-causative mutant SOD1, has made an enormous impact on progress of ALS/MND studies. Despite such advancement, recent studies have raised some

concerns about the use of mice with an inappropriately controlled genetic background, which sometimes lead to distorted experimental outcomes. Since the genetic backgrounds are associated with many physiological and pathological phenotypes, it is important to generate and use inbred or congenic lines of genetically-modified animals. However, there are still a limited number of such strains available for models of ALS/MND to date, and thus the effects of genetic background on ALS/MND phenotypes and their molecular basis are largely unknown.

Objectives: To investigate the effects of genetic background on phenotypes in ALS/MND mouse models, congenic lines of $SOD1^{H46R}$-tg, $SOD1^{G93A}$-tg, and Als2-knockout (KO) mice on two different genetic backgrounds; C57BL/6N (B6) and FVB/N (FVB) were generated.

Methods: Original $SOD1^{H46R}$-tg males (C57BL/6 $\times$ DBA/2) were bred with B6 females, resulting in B6 congenic $SOD1^{H46R}$ mice (>N10). Then, B6 congenic males were backcrossed with FVB females, generating FVB congenic $SOD1^{H46R}$ lines (N4-N9). $SOD1^{G93A}$ mice on two different genetic backgrounds were generated by crossing B6SJL-TgN(SOD1-G93A)1Gur males derived from Jackson Laboratories with either B6 or FVB females (N4-N9). Further, congenic lines of Als2-KO mice were generated by crossing F2 Als2-KO mice (129/Ola x C57BL/6) with either B6 or FVB mice (>N10).

Results: Both $SOD1^{H46R}$ and $SOD1^{G93A}$ mice exhibited progressive motor dysfunction and paralysis. Mean survivals of B6-$SOD1^{H46R}$ and B6-$SOD1^{G93A}$ mice were significantly longer than those of FVB-$SOD1^{H46R}$ and FVB-$SOD1^{G93A}$ mice, respectively. Kaplan-Meier analysis revealed that although there were no observable gender effects on the lifespan in B6 congenic SOD1-tg mice, females survived longer than males with a FVB background. On the other hand, all lines of Als2-KO mice were viable and fertile with no evidence for gross abnormalities. Kaplan-Meier analysis showed that both B6 and FVB congenic Als2-KO mice significantly lived longer than those with F2 animals. Interestingly, females exhibited longer lifespan than males in B6 but not FVB congenic Als2-KO lines.

Discussion and Conclusions: These findings indicate the presence of genetic modifier(s) for lifespan both in mutant SOD1-tg and Als2-KO mice. Intriguingly, a gender effect on the survival in mutant SOD1-tg and Als2-KO mice emerges in a genetic background-specific manner. These current observations support the notion that genetic background affects the physiological and pathological phenotypes in ALS/MND mouse models. Characterizations of the transgene copy numbers as well as the expression levels of the modified gene in these animals are currently underway.

P57 CHARACTERIZATION OF ALS/MND MOUSE MODELS CONCERNING MOTOR, BEHAVIORAL AND COGNITIVE DEFICITS

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Keywords: mouse model, dynein, behavior

Background: The phenotypes of animal models for Amyotrophic Lateral Sclerosis (ALS) and Motor Neuron Diseases (MND) are mainly defined by motor neuron degeneration and muscle paralysis. Concerning this the SOD1-G93A model is the best characterized mouse model.

Objectives: To analyze several mouse models which are described to develop phenotypes characterized by a predominant motor neuron pattern.

Methods: The motor, behavioural and cognitive deficits of animals with a mutation in the motor protein dynein (Cra1/+-mice) were investigated. Dynein acts together with the protein dynactin to play a crucial role in retrograde axonal transport. These two models were compared to a transgenic dementia mouse model carrying a human mutation in the protein tau (P301L-mice). The relation of mutations in SOD1-G93A or dynein (Cra1/+ mice) in MND mouse models and dementia was then examined.
By using a specific testing battery the spectrum of functional changes in the behaviour of transgenic animals concerning motor deficits, activity and cognition were defined. Only male SOD1-G93A-, Cra1/+-, Cra1/SOD1-G93A-, P301L/+-mice and age-matched non-transgenic littermates (all C3HB6 F1) at the age of 3 months were used. For further histological investigations, cryoslices of the quadriceps were made and stained with hematoxylin and eosin, to examine pathological changes of the muscle as well as the diameter of the muscle fibers.

Results: Besides distinct motor deficits the present results also indicated behavioral changes in Cra1/+-mice. In contrast to SOD1-G93A animals, these mice were hyperactive and showed deficits in coordination. Compared to wild type-littermates the spatial memory was normal in Cra1/+-mice, whereas SOD1-G93A mice learnt significantly faster. SOD1-G93A mice showed no changes in the behavior at this age. In histological examination of the quadriceps muscles animals with mutated SOD1-G93A showed neurogenic changes as well as a lesser diameter of muscle fibers.

Discussion and Conclusions: The deficits in behavioral tests, especially hyperactivity, cannot be explained by an exclusive motor pattern of vulnerability. In contrast, it is suggested that other regions of the CNS are also affected by the disease process. To explain the complex deficits of Cra1/+ mice, further neuropathological studies are clearly justified.

P58 USE OF TILLING TO DEVELOP A SOD1 ZEBRAFISH MODEL OF MOTOR NEURON DISEASE

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Keywords: SOD1, zebrafish, NMJ

Background: Mutations in the superoxide dismutase gene (SOD1) are known to cause familial amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) in humans. SOD1 is a soluble cytoplasmic and mitochondrial protein, which converts superoxide radicals to oxygen and hydrogen peroxide. In order to test potential therapies for MND, animal models of the disease are required. Zebrafish are an excellent model for human neurological diseases as they give plentiful transparent embryos, which are easy to manipulate and visualize. Furthermore, human and zebrafish SOD1 protein share 76% homology. Through TILLING (Targeting Induced Local Lesions IN Genomes), we have identified a zebrafish

carrying the T70I mutation in the SOD1 gene. The zfT70I mutation occurs in the zinc-binding loop of the SOD1 protein. The mutation results in change from a polar, nucleophilic threonine to a nonpolar, hydrophobic isoleucine amino acid. This change is predicted to have a deleterious effect on protein stability based on the I-Mutant-3.0 ΔΔG tool. Mutations in humans that affect Zn binding in this region affect amino acid polarity, similar to that in the zebrafish mutant. Based on these data it is predicted that the T70I zebrafish mutant is pathological.

Objectives: To generate homozygous and heterozygous T70I zebrafish and determine whether they develop MND-like symptoms. Biochemical, histological and behavioural phenotypes will be characterised.

Results: Several outcrosses of TILLED zebrafish have been carried out (to generation F5) in order to remove confounding deleterious mutations. Heterozygous and homozygous T70I mutants are viable and a normal Mendelian ratio of WT, heterozygote and homozygote embryos are present at 5 days post-fertilization (dpf). No overt behavioural abnormalities are observed in adult heterozygous mutants and homozygous mutants are being developed to determine if increased levels of mutant SOD1 can exacerbate the neuromuscular defects. Homozygous T70I mutant embryos have been generated and immunostaining has been performed with α-bungarotoxin (post-synaptic marker) and SV2 (pre-synaptic marker) in day 11dpf embryos. Transgenic zebrafish embryos carrying the G93R mutation show significant alterations in co-localization and intensity of the pre and post-synaptic markers at 11dpf. Preliminary histological data from NMJ staining in 11dpf T70I zebrafish larvae show a slight reduction in intensity of pre and post synaptic markers in the interseptal regions in the T70I homozygote as compared to their WT clutch-mates. Further evaluation of the NMJ characteristics is ongoing. The enzymatic activity of the T70I mutant has been characterised and the level of mutant protein has been measured at various time points and in multiple tissues, to determine if the mutation affects protein stability and clearance.

Discussion: The reduced NMJ staining in the 11dpf homozygous T70I zebrafish larvae is consistent with previous studies using transgenic mutant SOD1 zebrafish. Biochemical, histological and behavioural characterization of this novel mutant in zebrafish SOD1 gene will be presented.

P59 ZEBRAFISH EMBRYOS AS A MODEL FOR LOSS OF FUNCTION MUTATIONS IN THE PROGRANULIN GENE

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Keywords: zebrafish, progranulin, neurotrophic factor

Background: Frontotemporal lobar degeneration (FTLD) is a heterogeneous group of disorders characterized by neurodegeneration of prefrontal and anterior temporal cortex. Some forms of FTLD have features in common with amyotrophic lateral sclerosis (ALS). Recently, mutations in the progranulin gene were identified as a cause of familial FTLD. Since most mutations are loss of function mutations, reduced progranulin protein levels are thought to underlie the neurodegenerative process.

Objectives: To define whether progranulin has a neuroprotective function in the central nervous system.

Methods: Measurements of progranulin in the CSF by ELISA revealed that progranulin protein levels were reduced by more than 50% in patients with a Ser82fs mutation in the progranulin gene, but not in patients with FTLD without progranulin mutations, patients with ALS or Alzheimer's disease. To study the effect of reduced progranulin levels on the central nervous system, progranulin was knocked down in zebrafish embryos.

Results: Since the spinal motor neuron development of zebrafish embryos is very well characterized and can be easily visualized, it is an ideal model for gene knockdown studies using morpholinos. Knock down of granulinA and/or granulinB (two zebrafish homologues of progranulin) was obtained by injection of early zebrafish embryos with morpholinos. No obvious effect on central neurons or sensory neurons was observed, but the motor neuron axons were clearly shorter. This effect was most pronounced when both granulinA and granulinB levels were downregulated.

Discussion and Conclusions: These data support the hypothesis that reduced progranulin levels occur in patients with loss of function mutations in the progranulin gene and suggest that a shortage of progranulin may affect neuronal integrity.

P60 A MUTANT TDP-43 ZEBRAFISH EMBRYO MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: zebrafish, TDP-43, genetics

Background: Recent research into the genetic causes of inherited forms of amyotrophic lateral sclerosis (ALS), a fatal disease of selective motor neuron loss leading to muscle weakness and atrophy, has lead to the discovery of over thirty mutations within the TAR DNA-binding protein (TDP-43) gene. Further, TDP-43 has been identified as the major pathological protein of inclusions within the spinal cords of sporadic ALS patients.

Objectives: To establish whether a motor neuron phenotype similar to that previously reported following overexpression of another ALS causing gene, mutant SOD1 (1), is also present following injection of mutant TDP-43 RNA. To establish a small animal model of mutant TDP-43 expression to allow investigation into this form of the motor neuron disease.

Methods: Wild-type and mutant (A315T and M311V) TDP-43 RNA (50–100 ng/μl) were microinjected into 2–4 cell stage zebrafish embryos. At 30 hours post fertilisation (hpf) the embryos were dechoriated and fixed in 4% PFA. Whole mount fluorescence immunostaining of the embryos (anti-SV2) allowed visualization and scoring of the primary motor neuron axons exiting the spinal cord. The lengths of the first

five axons following the embryos yolksac were measured, as was the degree of aberrant axon branching along the full extent of the fish spinal cord.

Results: In comparison with non-injected embryos, those overexpressing wild-type TDP-43 alone exhibited shortened axonal length (decrease by 10 μm) and increased aberrant branching (25% affected) at higher RNA concentrations. However, injection of mutant TDP-43 produced a more pronounced shortening (decreases of 18 and 21 μm for A315T and M311V respectively) of motor axons and more frequent aberrant branching (up to 60% affected). Both measures of axopathy were dose-dependent and the axopathy was significantly worse in zebrafish injected with mutant than wild-type TDP-43 RNA ($P < 0.029$).

Discussion: This motor neuron phenotype is similar to that in response to mutant SOD1 described previously, although the axopathy in response to wild-type RNA is specific for TDP-43. This latter point raises the possibility that over-expression of wild type TDP in sporadic ALS patients may also be disease causing.

Conclusions: With the development of this TDP-43 model factors that may rescue the phenotype can be searched for through simultaneous knock-down of other disease related genes (via morpholino injection) or overexpression of other RNAs, prior to expansion into more complex and time consuming models such as mutant-TDP expressing mice and ultimately patients.

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P61 NOTCH SIGNALING IN MOTOR NEURON REGENERATION IN THE LESIONED SPINAL CORD OF ADULT ZEBRAFISH

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Keywords: notch, zebrafish, spinal cord

Background: *De novo* neuronal production in the adult mammalian spinal cord following spinal cord injury is highly inefficient and the functional deficits are permanent. Motor neurons lost in progressive and often fatal motor neurone diseases are also not replaced. By contrast, adult zebrafish (Danio rerio), which demonstrate functional regeneration after complete transection of the spinal cord, are capable of regenerating lost motor neurons (1). Interestingly, neuronal differentiation in the lesioned spinal cords of adult rats may be blocked by notch signalling (2).

Objectives: To elucidate the role of the notch pathway during successful motor neuron regeneration in the lesioned zebrafish spinal cord, because this could reveal aspects of notch activity that may promote motor neuron regeneration.

Methods: *In situ* hybridisation and semi-quantitative RT-PCR was used to find the key components of notch signalling in the lesioned spinal cord of zebrafish. Notch activity was manipulated in the lesioned spinal cord by genetic and pharmacological means to find changes in motor neuron regeneration.

Results and Discussion: Gene expression analysis of components of the notch pathway reveals that the notch1a and notch2 receptors, the downstream target gene, her4 and the notch augmenting gene musashi1 are upregulated in ventricular progenitor cells at two weeks post lesion compared with the unlesioned spinal cord. However, expression of the notch ligands deltaD, dll-4 and jagged2 was undetectable by *in situ* hybridisation. It was found that a heat-shock inducible dominant active form of notch (3) can be overexpressed in adult spinal stem cells. Injection of gamma-secretase inhibitor DAPT, which blocks notch activity, reduces expression of the her4 target gene. Effects of these manipulations on motor neuron regeneration are currently under investigation.

Conclusions: Upregulation of notch1a, notch2, her4 and musashi1 mRNA expression in the lesioned spinal cord of adult zebrafish suggest roles of notch signaling in successful adult motor neuron regeneration.

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THEME 3 *IN VITRO* EXPERIMENTAL MODELS

P62 STRUCTURAL CHANGES TO MONOMERIC CUZN SUPEROXIDE DISMUTASE CAUSED BY THE FAMILIAL ALS ASSOCIATED MUTATION A4V EXAMINED VIA MOLECULAR DYNAMICS SIMULATIONS

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Keywords: superoxide dismutase, molecular dynamics, A4V

Background: Familial ALS (fALS), has been linked to over 100 different point mutations scattered throughout the Cu-Zn superoxide dismutase protein (SOD1). The disease is likely due to a toxic gain of function caused by the misfolding, oligomerization and eventual aggregation of mutant SOD1, but it is not yet understood how the structurally diverse mutations result in a common disease phenotype.

Objectives: To identify structural changes to monomeric SOD1 caused by the fALS associated mutation A4V that may lead to oligomerization of the mutant protein.

Methods: Simulations of apo WT and A4V SOD1 were performed using the *in lucem* molecular mechanics (ilmm) simulation software, including all hydrogens and explicit flexible three-center waters. Periodic boundary conditions were employed to minimize edge effects. The microcanonical ensemble was employed. Three 60 ns simulations were run for each of the WT and A4V apoprotein at 310 K, giving approximately 180,000 structures each for analysis.

Results: The simulations revealed that during its course the A4V mutant protein undergoes larger structural changes than WT when measured by Cα RMSD, solvent accessible surface area, radius of gyration and CONGENEAL structural dissimilarity score. Residues that are normally involved in the dimer interface are perturbed in the mutant and the residues of β8 form a tighter association with those of β1. The zinc binding residues move further apart from each other in the mutant and a non-native α-helix forms in the zinc binding loop of the wildtype protein. There is a loss of β-sheet beginning in strands β5 and β6.

Discussion: Perturbation of the dimer interface residues may prevent easy dimerization and destabilize the dimer which is a known effect of the A4V mutation. The non-native helix that forms in the demetallated wildtype simulations may serve to stabilize the local structure for future zinc binding, while the increased distances of the zinc binding residues in the mutant may hinder this. The loss of β structure exposes edge strands that may lead to eventual edge-edge oligomerization.

Conclusions: Simulations show that the mutant protein is less stable than the WT protein overall, with shifts in residue-to-residue contacts that lead to destabilization of the dimer and metal-binding sites and stabilization of non-native contacts that leads to a misfolded state. These findings provide a unifying explanation of disparate experimental observations, allow a better understanding of alterations of residue contacts that accompany loss of SOD1 structural integrity and suggest sites where compensatory changes may stabilize the mutant structure.

P63 CHARACTERIZATION OF AS-ISOLATED G93A HSOD1 MUTANT FROM TRANSGENIC RAT SPINAL CORD: ZINC-DEFICIENT ENZYME CORRELATES WITH DISEASE SEVERITY

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Keywords: thiol, zinc isotopes, copper

Background: Mutations to SOD1 have been known to cause ALS for over 16 years, but the mechanism of neurotoxicity remains unknown. *In vitro* studies with recombinant SOD1 mutants have been shrouded in controversy as to which properties are "real" and which are related to the necessarily artificial conditions used to express and purify them. This study purified G93A and wild-type (WT) hSOD1 from transgenic (Tg) rat spinal cord using very mild conditions, so that the true "as isolated" properties of a disease-causing mutant could be unequivocally established.

Objectives: To compare properties of the human G93A mutant enzyme purified from spinal cord of Tg rats before and during disease, with those of WT hSOD1 from Tg rats, as well as recombinant G93A obtained from E. coli. The properties of G93A "as isolated" from spinal cord may provide clues as to the toxic mechanism of SOD1 mutants and allow us to better assess the suitability of recombinant enzymes to test various toxic hypotheses.

Methods: Spinal cords from Tg rats (four time points) were homogenized and clarified tissue supernatants injected into a polymeric, reversed-phase preparative HPLC column maintained and eluted at pH 7.4. The fraction corresponding to native homodimer was collected and overloaded onto an SDS-PAGE gel to assess purity. For each sample ($n = 3$ for each condition), Zinc (Zn), Copper (Cu), reduced thiol (-SH) content, Zn isotope uptake and specific dismutase activity were determined and normalized to enzyme subunit based on rigorous determination of protein concentration.

Results: SDS-PAGE gels revealed that the one-step HPLC purification procedure yielded >99% pure G93A from rat cord within 20 min of tissue homogenization. Analysis of ultrapure enzyme revealed ~50% of the maximal content of Zn and Cu. Zn isotope uptake (^{67}Zn and ^{65}Zn) revealed a consistent increase in Zn-deficient enzyme as the disease worsened. -SH content of native enzyme was ~0.2/subunit and increased to ~1.2 upon denaturation. Specific activity of rat cord G93A was ~4,000 units/mg, which is equivalent to ~8,000 units/mg for copper-saturated enzyme.

Discussion: The results are consistent with the surface accessible cysteine (Cys111) being very labile and existing in the oxidized state, whereas Cys6 is reduced. The ratios of Zn and Cu per subunit are consistent with one metal ion per dimer. Limiting amounts of CCS and Cu could explain the low Cu levels (when SOD1 is grossly overexpressed), but not the low levels of Zn. The fraction of G93A mutant which is Zn-deficient increased steadily with disease severity, with as much as 12% of the native dimer existing in this form,

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despite the dilutional effect resulting from use of whole cord homogenates. This finding has implications for neurotoxicity, given that Zn-deficient enzyme is both pro-aggregatory and pro-oxidant.

P64 SOD1 FIBRILLATION MECHANISM RELEVANT TO FAMILIAL FORM OF ALS

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Background: Dominant mutations in Cu, Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis (fALS). SOD1 polypeptide becomes stabilized and enzymatically active after post-translational modifications including Cu and Zn binding and intramolecular disulfide formation (1). A growing body of evidence suggests that fALS-causing mutations destabilize the native structure of SOD1, leading to aberrant protein interactions for aggregation (2). While accumulation of insoluble mutant SOD1 in the affected tissues is a common pathological hallmark of SOD1-related fALS, it should be also noted that some phenotypes such as disease duration are dependent upon the type of mutations in a SOD1 protein. A mutation-dependent cytotoxicity of SOD1 will thus be an important factor to understand the pathomechanism of SOD1-related fALS.

Objectives: To reveal a molecular mechanism describing mutation-dependent phenotypes in SOD1-related fALS, by characterizing the biochemical, morphological and cytotoxic properties of fibrillar aggregates of mutant SOD1 proteins.

Methods: Recombinant wild-type (WT) and mutant (L144F and I149T) SOD1 proteins were prepared. A fibrillation process of SOD1 was monitored by Thioflavin-T fluorescence (3). Regions responsible for aggregate formation in a primary sequence of SOD1 (called as aggregation core) were determined by a mass spectrometry and morphologies of the SOD1 aggregates were characterized by electron microscopy. To examine the cytotoxicity of SOD1 aggregates, an MTT assay was performed using mouse neuroblastoma cells, Neuro2a, which were incubated with *in vitro* SOD1 aggregates.

Results: Apo-SOD1 without disulfide is the most facile state for formation of insoluble aggregates (3). Although aggregates of both WT and mutant SOD1s possess fibrillar morphology, differences exist among WT, L144F and I149T fibrils especially in the surface roughness and the length. An aggregation core in WT fibrils covers 1–28, 90–116 and 136–152 in the SOD1 primary sequence, but 136–152 is not involved in a core of I149T aggregates. WT and L144F aggregates show minimal cytotoxicity, but significantly reduced cell viability was observed when I149T aggregates were incubated with Neuro2a cells.

Discussion and Conclusions: SOD1 forms fibrillar aggregates upon demetallation and disulfide reduction (3). The results presented here indicate mutant-specific properties of aggregate structure as well as cytotoxicity. These studies thus imply a new pathomechanism of fALS in which mutation-dependent properties of SOD1 aggregates modulate degree of disease phenotypes.

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P65 BIOCHEMICAL ANALYSIS AND FUNCTIONAL CHARACTERISATION OF PURIFIED MUTANT HOMO- AND HETERODIMERIC SOD1 PROTEINS

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Keywords: SOD1 homodimer, SOD1 heterodimer

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease leading to the loss of motor neurons in the spinal cord and in the motor cortex. An early observed association of point mutations in the gene for Cu/Zn superoxide dismutase (SOD1) with some familial cases of ALS provided the basis for the establishment of a variety of *in vivo*- and *in vitro*-models. This ubiquitously expressed enzyme catalyzes the dismutation of the free radical superoxide into oxygen and hydrogen peroxide and thereby removes superoxide from the cell. A functional active SOD1 enzyme consists of two SOD1 subunits. Since nearly all SOD1 mutations are dominantly inherited, patients with a mutation in the SOD1 gene express wild-type as well as mutant SOD1 proteins. These SOD1 proteins can form dimers containing two wild-type or two mutant subunits (homodimers) or a wild-type and a mutant subunit (heterodimer). To examine the biochemical and functional behaviour of these different composed dimmers, SOD1 dimer constructs were generated where two SOD1 subunits are molecularly attached to each other by a short polypeptide. This recently demonstrated that heterodimers in a cellular and a C. elegans model displayed at least the same toxicity. In the case of SOD1(G85R) heterodimers, even an increased toxicity was found as compared to mutant homodimers. The purpose of this study is to determine the structural and functional properties of homo- and heterodimeric SOD1 proteins.

To purify SOD1 dimer constructs, a procaryotic expression system with BL21 (DE3)pLysS competent cells was used. After gaining high expression levels, 6xHis-tagged dimer constructs were purified with Immobilized Metal ion Affinity Chromatography (IMAC). Dismutase activity of all purified dimer constructs was shown in activity assays. Coomassie blue stainings showed that all mutant homodimeric proteins could be found in insoluble inclusion bodies while heterodimeric SOD1 proteins remained soluble like the wild-type homodimeric control. This result confirms previous observations that the SOD1 homodimers reveal a greater tendency to aggregate than their heterodimeric analogs (1). Proteinase K assays revealed that some SOD1 mutant proteins are more sensitive to Proteinase K than others. Nonetheless, a wild-type subunit seems to stabilize the dimeric conformation for SOD1 heterodimers so that heterodimeric proteins are less sensitive to Proteinase K digest than homodimeric SOD1.

In SOD1 heterodimers a wild-type subunit seems to have a great influence on the stability and aggregation tendency of SOD1 dimers. It also might influence the severity and progression of the disease in human patients.

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P66 THE MECHANISM OF CLEAVAGE AND CELLULAR LOCALIZATION OF TDP-43 AS A CAUSATIVE PROTEIN OF ALS

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Keywords: TDP-43, cleavage, localization

Background: TAR DNA-binding protein TDP-43 (encoded by TARDBP gene in chromosome 1p36.22) was identified as the major constituent of ubiquitin-positive cytoplasmic inclusions in sporadic ALS and frontotemporal lobar degeneration (FTLD) (1).
TDP-43 is mainly localized to the nucleus and implicated in the splicing of the cystic fibrosis transmembrane receptor (CFTR) by recruiting hnRNP, which is involved in mRNA biogenesis. Although abnormal deposition of wild-type TDP-43 is observed in the sporadic ALS lesion, approximately 30 missense mutations, most of which are located in the C-terminal of the protein, are identified in both sporadic and familial ALS patients (2). Phosphorylated 25 kDa C-terminal cleaved fragments accumulate and aggregate in the motor neurons of ALS patients, and the finding may be relevant to motor neuron degeneration in ALS (3). However, the cleavage mechanism of TDP-43 and the fate of cleaved N- and C-terminal fragments remain unknown.

Objectives: To elucidate the cleavage and localization mechanism of TDP-43 by biochemical approach.

Methods: Cultured cells (neuronal or non-neuronal cells), expressing dual tagged TDP-43 were used in order to monitor the N- and C-terminal fragments. Wild-type and TDP-43 mutants were transiently expressed in these cells, cleavage and localization of TDP-43 were analyzed. In this system, the cleavage of TDP-43 was observed both in the nucleus and the cytosol and more efficiently induced in the ALS-linked mutants. Moreover, TDP-43 fragments cleaved in the nucleus were different from the ones in the cytosol. When TDP-43 was cleaved in the nucleus, both fragments aggregated in the nucleus. Interestingly, N-terminal fragments of TDP-43 cleaved in the cytosol were localized to the nucleus in the nuclear localization signal (NLS) independent manner and this fragment did not form aggregates in the nucleus. On the other hand, C-terminal fragment was localized to the cytosol and formed aggregates.

Discussion and Conclusions: TDP-43 can be cleaved in the nucleus and cytosol by different mechanism. TDP-43, however, needs to be cleaved in the cytosol to prevent the formation of nuclear aggregates. Taken together, these results suggest that it is essential for N- and C- terminal fragments of TDP-43 to be rapidly transported into the nucleus after cleavage in the cytosol by distinct mechanisms to avoid the formation of aggregates in nucleus and/or cytosol. Accordingly, an investigation is underway to discover how each TDP-43 fragment is transported to the nucleus after cleavage. Moreover, this study aims to identify the enzyme(s) that cleave TDP-43 in the nucleus and cytosol. The detailed results will be reported at the Symposium.

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P67 MUTANT SOD1 AGGREGATION IN A CELL MODEL OF ALS: TDP-43 LOCALIZATION, LEVELS OF CO-EXPRESSED SECRETORY AND MEMBRANE GLYCOPROTEINS AND EFFECT OF TREHALOSE

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Keywords: glycoprotiens, TDP-43, trehalose

Background: A familial form of the neurodegenerative disease amyotrophic lateral sclerosis (ALS), is caused by dominant mutations in Cu, Zn superoxide dismutase (SOD1). *In vivo*, protein inclusions rich in mutant SOD1 have been found in tissues from familial ALS (FALS) patients, mutant SOD1 animals and cellular models. More recently, aggregation of the TAR DNA binding protein of 43 kDa (TDP-43) has been found in patients with sporadic ALS but not in FALS patients with mutant SOD1 pathology. Mutant SOD1 overexpression in cell models of FALS has been shown to impair intracellular trafficking of proteins. Small molecules, such as trehalose, have been used to decrease protein aggregation characteristic of neurodegenerative diseases.

Objectives: To study the following characteristics of a cell model of FALS; the NSC-34 cell line overexpressing mutant $SOD1^{G93A}$, localization of endogenous TDP-43; levels of co-expressed secretory glycoproteins β-trace protein (β-TP), erythropoietin (EPO), and of the cell adhesion molecule L1 as well as the effect of trehalose on SOD1 aggregation.

Methods: The mouse motor neuron-like NSC-34 cell line overexpressing human mutant $SOD1^{G93A}$ fused to the EGFP tag at the C-terminus was used as a cell model of familial ALS. Protein aggregates were detected by confocal fluorescence microscopy. Protein analysis was performed by Western blot.

Results: NSC-34 cells transiently overexpressing mutant $SOD1^{G93A}$-EGFP formed protein aggregates that were immunoreactive to ubiquitin. TDP-43 was predominantly detected in the nucleus of the cells as in healthy conditions and no large aggregates were detected.
Cells expressing mutant SOD1 exhibited lower levels of the secretory glycoproteins b-TP and EPO in the supernatants, which were not due to intracellular accumulation. The deglycosylation profile of β-TP with endoglycosidase H and peptide N-glycosidase F showed that it contained complex-type N-glycans. Furthermore, the N-glycosylation type was similar for b-TP obtained from wt or mutant SOD1 expressing cells. A lower amount of the membrane glycoprotein L1 was also observed in the cellular extract and shedded to the supernatant from mutant SOD1 expressing cells.
The effect of trehalose on mutant SOD1 aggregation was investigated. The percentage of cells containing SOD1 aggregates was statistically significant and decreased in the presence of 10 mM trehalose. In agreement a detergent insolubility assay showed that the amount of insoluble mutant SOD1 was decreased in the presence of trehalose. On the other hand, the addition of trehalose *in vitro* to preformed aggregates did not promote their solubilization.

Discussion and Conclusions: There was lack of TDP-43 pathology in a cell model of SOD1 FALS similar to that previously described for patients.
Mutant SOD1 overexpression led to decreased levels of secretory and plasma membrane glycoproteins. It is possible

that mutant SOD1, by triggering ER stress, causes reduced synthesis of those glycoproteins. Trehalose prevented *de novo* formation of aggregates in the FALS cell model.

P68 SOD1 EFFECT ON CELL CYCLE PROGRESSION IN AN ALS MODEL

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Keywords: cell cycle, mutant SOD1, SH-SY5Y

Background: Neuroblastoma cell line SH-SY5Y (SH) transfected with the gene encoding for mutant SOD1 is a well-known cellular model of Amyotrophic Lateral Sclerosis (ALS). Alterations of G1 to S cell-cycle progression regulators like CDKs and E2F have been previously described in ALS (1). Bcl2 can modulate cell cycle re-entry from quiescence (G0) regulating p27 expression and it also controls Reactive Oxygen Species (ROS) intracellular levels depending on its phosphorylation. p27 is a member of the Cip/Kip family which inhibits cell cycle progression from G1 to S. Stathmin (Op18) has a key role in cell cycle progression and its expression has been seen altered in the SH cellular model(2) and in mutant SOD1 transgenic mice (3). Although ROS are commonly toxic at specific concentrations they have a role in cell cycle progression (4).

Objectives: To investigate the possible role of mutated SOD1 in the cell cycle. Cell cycle position, expression of cell cycle regulators p27, Op18, Bcl2 and the activity of Op18 and Bcl2 evaluated by phosphorylation on Ser16 and Ser70 respectively, have been studied in the SH-SY5Y cell line, transfected with wild-type (WT) or mutant SOD1 (G93A).

Methods: ROS levels and cell cycle progression were assessed by flow cytometry; differences between the three lines were highlighted by synchronizing them with serum-deprivation protocol (5). Protein expression was evaluated by Western blotting. Op18 and Bcl2 phosphorylation was studied with In-Cell Western Assay (Odyssey®-Infrared Imaging System).

Results: Growth curves showed a higher proliferation of G93A cells compared to SH and WT. After synchronization, flow cytometry analysis confirmed a high distribution of WT in G1 phase, whereas G93A was more distributed in S and G2/M phases ($P < 0.05$). p27 expression and Op18 phosphorylation were consistent with cell cycle results: p27 was higher in WT than in G93A and Op18 was less phosphorylated in WT compared with G93A ($P < 0.05$). There were no differences in Op18 expression. There were no significant differences in ROS and Bcl2 phosphorylation level among the three lines.

Discussion: These data indicate that mutant SOD1 alters cell cycle progression in an ALS cellular model. From this data, cell cycle alterations are not linked to ROS level or Op18 phosphorylation suggesting instead a primary role of Bcl2/p27 pathway in the mutant-linked abnormalities. Experiments are in progress to investigate whether Bcl2 regulates p27 modulating its expression or through a negative control of its degradation.

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P69 ESTABLISHMENT OF A HUMAN EMBRYONIC STEM CELL-DERIVED FAMILIAL ALS MODEL

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Keywords: human embryonic stem cell, SOD1, ubiquitin

Background: Generation of amyotrophic lateral sclerosis (ALS) models is an important subject for investigating disease mechanisms and pharmacological applications. In the past 10 years or more, transgenic mice carrying a mutant superoxide dismutase 1 (SOD1) gene have been used as familial ALS (FALS) models. Although SOD1 mutant transgenic mice show similar phenotypes to human FALS, it is difficult to compare between transgenic mice and human patients because of different species and body sizes. Furthermore, drug response in animal models is not always the same as that in human patients. Animal disease models are certainly still required, however, human cell-based *in vitro* assay systems are also demanded because such systems could be helpful to obtain novel insights to disease mechanisms and overcome a decade issue of species difference.

Objectives: To establish an *in vitro* FALS model from human embryonic stem cells (hESCs) expressing the mutant SOD1 gene to evaluate disease phenotypes. To check whether cell death occurs in mutant SOD1 spinal motor neuron (sMN).

Methods: SOD1 transgenic hESC clones were generated by transfection of expression vectors containing representative FALS mutant, SOD1 G93A or wildtype SOD1. After neural induction of each transgenic clone by Noggin treatment for seventeen days, sMN differentiation was induced by all-trans retinoic acid (ATRA) and sonic hedgehog (Shh) for one week. Cell biological and pathological analyses were performed to evaluate FALS model culture.

Results: TUNEL assays were carried out within 7 days after sMN differentiation by ATRA and Shh treatment. TUNEL-positive apoptotic cell death was specifically observed in sMNs derived from SOD1 G93A mutant hESCs whereas wildtype SOD1 hESCs did not show a significant change of sMN death compared to the parent hESC line. Ubiquitin aggregations were detected in cells expressing SOD1 G93A

among mixed populations of sMN differentiation culture conditions.

Discussion and Conclusions: This established hESC-derived FALS *in vitro* model showed similar sMN specific cell death phenotype to previous reports that analyzed animal models or hESC-derived models. Future pathological analysis is of importance to evaluate this *in vitro* model compared to human patients. This FALS model can be applied as drug screening/evaluation assays, for evaluating the prevention of apoptosis. sMN populations have already been isolated from mixed culture. Further analysis for isolating sMN or combining sMN with other cells will be able to determine whether a cell-autonomous or non-cell autonomous effect influences sMN specific cell death. The techniques for both differentiation of sMNs from pluripotent stem cells and evaluation of FALS pathology can also be applied to investigations for personalized medicine using patient tissue-derived induced pluripotent stem cells.

P70 INDUCED PLURIPOTENT STEM CELLS FROM SOD1G93A MICE

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Keywords: SOD1G93A mice, induced pluripotent stem (iPS) cells, neuron

Background: SOD1G93A mice are a widely accepted model for ALS research, as they develop clinical symptoms similar to those seen in ALS patients. They also provide a uniquely advantageous model system for investigating mechanisms of disease in neurodegeneration. Induced pluripotent stem (iPS) cells are indistinguishable as compared to embryonic stem (ES) cells in proliferative and developmental potential.

Objectives: To investigate whether the mutant expression of SOD1 influences nuclear reprogramming in the generation of iPS cells. To investigate whether iPS cells from SOD1 mice and C57BL/6 can differentiate into neural tissues.

Methods: Fibroblast cells from adult C57BL/6 and SOD1G93A mice were isolated. Retroviral infection was performed for expression of Oct4, Sox2, Klf4, c-Myc and iPS cells were then induced. Cell cultures were performed as described by others. Embryoid bodies and teratoma formatted and differentiated into endoderm, mesoderm/cardiomyocyte, and ectoderm/ neurons. The differentiated cells were collected for RT-PCR analysis. All experiments were performed in three independent runs. Immunofluorescence was used for detecting nestin, Tuj1, GFAP, MAP and TH after neural induction. Statistical significance was assessed by Student's t test or analysis of variance with Tukey's multiple comparison test.

Results: iPS cell clones exhibited and maintained morphology distinct from the original human fibroblasts and resembled hES cells for at least 14 passages in culture. iPS clones exhibit hES-like properties, including high AP activity, and positive staining for Sox2, Oct4, TRA-1-60 and SSEA-4. Staining intensity for all markers tested was similar to that observed with the well-characterized hES cell. The original fibroblast cell line was negative for these markers. RT-PCR analysis confirmed expression of the genes essential for hES cell maintenance. None of these genes were expressed in the original fibroblasts. Teratomas were formed 4–6 weeks after the iPS cell injection. HE and immunostaining of tumor sections demonstrated the presence of cell types of all the 3 germ layers, including neural and non-neural ectoderm, pigmented epithelium, smooth muscle and fat tissue (mesoderm), airway and gut-like epithelial tissues.
iPS cells could be continually passaged using a chopping method that avoids losing cell-cell contact known to be important for maintaining both neural and embryonic stem cell proliferation. These cultures were then dissociated and plated onto laminin-coated coverslips. iPS spheres generated nestin-positive cells indicative of a neural stem cell phenotype. On further differentiation, Tuj1, TH positive neurons and GFAP positive astrocytes were also found. iPS spheres were simple to expand, remarkably stable over time and maintained the ability to produce neural progeny for more than 20 passages.

Conclusions: iPS cells from SOD1 mice were obtained which can be used as a model to investigate the specific pathology seen in ALS. As such, it represents a promising resource to develop new therapies.

P71 REAL-TIME IMAGING REVEALS DEFECTS OF FAST AXONAL TRANSPORT INDUCED BY DISORGANIZATION OF INTERMEDIATE FILAMENTS

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Keywords: axonal transport, neurofilament, peripherin

Background: Many human neurodegenerative disorders, including amyotrophic lateral sclerosis, Parkinson's disease, Charcot-Marie-Tooth disease and giant axonal neuropathy, are characterized by the abnormal accumulations of intermediate filaments (IF). Several evidences suggest that disorganization of the neuronal IF network may be directly involved in neurodegeneration. However, the exact molecular mechanisms underlying the deleterious effects of IF disorganization are still unclear.

Objectives: To test the hypothesis that changes in the organization of IF can alter the fast axonal transport machinery either through levels of motor proteins or blockage of cargo movement.

Methods: Time-lapse imaging was used to analyse the mitochondrial and lysosomal axonal transports in cultured dorsal root ganglion (DRG) neurons from different mouse models with various abnormalities of the endogenous IF network. Among these models are NFL knockout mice (NFL −/− mice), which are characterized by the absence of axonal neurofilaments; mice overexpressing peripherin (Per mice) that develop perikaryal and axonal IF aggregates that were associated with the death of motor neurons *in vivo* and also of DRG neurons *in vitro*; and finally Per;NFL −/− mice in which the onset of peripherin-mediated disease is precipitated.

Results: Unexpectedly, a net retrograde transport of mitochondria was detected in Per;NFL −/− neurons in contrast

to a net anterograde transport of these organelles in Wt, NFL −/− and Per neurons. Consistent with this, a reduced content of mitochondria was observed in distal sciatic nerve from 1-year-old Per;NFL −/− mice, concomitant with a misdistribution of molecular motors along this nerve. The depletion of axonal neurofilaments in NFL −/− and Per;NFL −/− neurons induced longer and more persistent movements of mitochondria and lysosomes in both anterograde and retrograde directions. This indicated that the endogenous neurofilament network tends to slow down fast axonal transport. In contrast, the overexpression of peripherin alone did not induce major alterations of fast axonal transport.

Discussion and Conclusions: These results reveal an involvement of IF in fast axonal transport and suggest that disorganization of IF characteristic of human neurological disorders may contribute to neurodegeneration by altering the fast axonal transport of organelles. They also suggest that the neurofilament network can slow fast axonal transport by increasing the frequency of pauses and reducing the amplitude of the organelle movements in the axon. An unexpected finding was that an increase of peripherin concomitant with NFL deficiency can provoke a net retrograde transport of mitochondria. This phenomenon may contribute to pathogenic changes in some neurodegenerative disorders with IF protein accumulations.

P72 MICROFLUIDIC INVESTIGATION OF EXCITOTOXIN INDUCED ALS-LIKE AXONAL DEGENERATION

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Keywords: excitotoxicity, axon, microfluidic

Background: Recent data supports the notion that axon dysfunction is a significant factor in causing the symptoms of ALS prior to the death of motor neurons. Degeneration of distal lower motor neuron axons has been reported to be among the first degenerative changes in ALS mouse models. Distal degeneration of axons in the tracts of the spinal cord in these models has also been detected (unpublished data). This degeneration involves abnormal localization of the intermediate filament protein alpha-internexin to the lateral and ventrolateral funiculus, in addition to anterior corticospinal tract. Previous investigations have determined that excitotoxicity, a likely causative factor in ALS, can result in distal axonopathy resembling ALS pathology. However, the site of primary degeneration may not be indicative of the site of primary toxicity.

Objectives: To utilize microfluidic technology and cell culture models of ALS pathology to investigate the role of the axon and somatodendritic compartments in excitotoxin induced distal axonopathy.

Methods: Cortical neurons were obtained from embryonic day 15 C57Bl/6 mice and plated into one side of a microfluidic chamber. These chambers allow the growth of axons through 10×3 μm channels, with the exclusion of cell bodies. Complete microfluidic isolation of axonal and somatodendritic compartments was maintained by differential hydrostatic pressure between channels. To investigate the source of toxicity excitotoxins were applied to cell bodies or axons. Results were analysed using imunocytochemical techniques. In order to directly model the potential motor neuron-muscle interactions that occur *in vivo,* these techniques will be applied to lower motor neurons grown on a bed of astrocytes with axons extending to a muscle cell monolayer. Lower motor neurons were obtained from embryonic day 13 mouse spinal cords and purified on an optiprep gradient. Astrocyte and muscle feeder layers were derived from postnatal day 3 mouse pups.

Results: Immunocytochemical analysis of cultured cortical neurons grown in microfluidic chambers have confirmed that MAP2 immunoreactive dendrites do not extend to the axon compartment. These investigations using cultured spinal motor neurons have demonstrated that chronic application of low concentrations of kainic acid (25 μM) to spinal motor neurons results in a distal axonopathy consisting of distinct accumulations of neurofilament proteins and associated with a mislocalization of de-phosphorylated neurofilament epitopes to the distal axon. Microfluidic cell culture models of lower motor neurons involving neuromuscular synapses will be used to investigate the source of toxicity in distal axonopathy.

Discussion: These experiments will provide data to aid in the elucidation of mechanisms of axon degeneration relevant to ALS and will be important in the development of potential therapeutic agents involved in axon protection.

P73 EFFECT OF SEX STEROIDS ON MITOCHONDRIAL GENE EXPRESSION IN SPINAL CORD ASTROCYTES AND NEURONS

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Keywords: sex hormones, mitochondria, spinal cord

Background: Amyotrophic lateral sclerosis (ALS) is a progressive disease characterized by degeneration of upper and lower motor neurons. Pathological changes in other local cell such as astrocytes have been often neglected as a secondary and unspecific response to proceeding neurodegeneration. Gender is a well-known risk factor for ALS with a male/female ratio of 2:1. The role of sex hormones for ALS is also suggested by the observation that women who develop ALS are characterized by an early and late onset of menarche and menopause, respectively.

Objectives: To assess the role of sex hormones in the regulation of mitochondrial function in spinal cord neurons and astrocytes since sex hormones are well-established neuroprotective factors in the brain and spinal cord.

Methods: Transcriptional activity of mitochondria-encoded catalytic subunits of proton-translocating respiratory chain complexes was analyzed by real-time RT-PCR in cultured mouse embryonic spinal cord neurons and neonatal astrocytes. To assure the feasibility of a sex-specific regulation, male and female cells were cultured separately.

Results: The application of 17ß-estradiol but not testosterone significantly increased the expression of four investigated catalytic subunits (ND1, CytB, Cox2 and ATP6) in spinal cord neurons. The estrogen receptor antagonist ICI 182780 abrogated estrogen effects on neurons suggesting classical genomic signaling. In female astrocytes, testosterone increased the expression of ND1 and CytB. No steroid-dependent regulation of subunits was observed in male

astrocytes. The androgen receptor antagonist cyproterone acetate was ineffective in abolishing testosterone effects.

Discussion and Conclusions: Our results show that sex steroids are able to increase the expression of mitochondrial-encoded subunits of the respiratory chain in the spinal cord. Estrogen effects were more pronounced in neurons compared to astrocytes. Astrocytes revealed a gender-specific regulation. These data suggest a putative protective role for gonadal steroid hormones during the course of ALS and other motor neuron diseases and may explain, in part, the existing sex prevalence in the onset and severity of these disorders. The potency of steroid-mediated neuroprotection in the spinal cord remains to be further scrutinized in relevant animal models such as ALS and other motor neuron degeneration mouse models.

P74 THE PRO-INFLAMMATORY ACTION OF MICROGLIAL P2 RECEPTORS IS ENHANCED IN SOD1 MODELS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: extracellular ATP, purinergic receptors, microglia

Background: Common theories to explain the pathogenic mechanisms of ALS include activation of microglia, responsible for the release of pro-inflammatory factors. Extracellular ATP constitutes a well recognised neuron-to-microglia alarm signal through purinergic P2X and P2Y receptors, whose activation evokes many pro-inflammatory functions of microglia, such as chemotaxis, phagocytosis, production and release of cytokines/chemokines. The purinergic system is in fact critical in degenerative diseases involving neuroinflammatory components such as ischemia, Alzheimer's disease, experimental autoimmune encephalitis and prion infection. This may also be the case for ALS, where for instance $P2X_7$ receptor subunit was found to be significantly elevated in active microglia from post-mortem spinal cords of patients and in a rat model of familial ALS (fALS) as well.

Objectives: To investigate how the expression of mutant Cu, Zn superoxide dismutase (SOD1) model of fALS, affects P2 receptor-mediated pro-inflammatory microglial properties.

Methods: Primary and immortalised microglial cells from mutant SOD1 mice were used to investigate several aspects of activation by purinergic ligands and to analyse the overall effect of such stimulation on the viability of NSC-34 and SH-SY5Y neuronal cell lines.

Results: An up-regulation of $P2X_4$, $P2X_7$ and $P2Y_6$ receptors and a down-regulation of ATP hydrolysing activities in mutant SOD1 microglia was observed. This potentiation of the purinergic machinery reflects an enhanced sensitivity mainly to 2′-3′-O-(benzoyl-benzoyl) ATP, a $P2X_7$ receptor preferential agonist and translates into deeper morphological changes, enhancement of tumour necrosis factor α and cyclooxigenase-2 content. Such potentiation then selectively commits microglia expressing mutant SOD1 to exert toxic effects on neuronal cell lines.

Conclusions: The purinergic activation of microglia may constitute a new route involved in the progression of ALS to be exploited to halt the disease.

P75 GLUTAREDOXINS AND MUTANT SOD1S: A STUDY IN NEURONAL CELL MODELS

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Keywords: mitochondria, SOD1, glutaredoxin

Background: Intracellular SOD1 aggregation is a typical hallmark of pathology in several ALS models as well as in patients. SOD1 aggregates have been found in the cytosol and mitochondria of neuronal cells from ALS mouse models. Several *in vitro* data show that the propensity of SOD1 to form oligomers depends upon the redox state of specific cysteine residues. Moreover, *in vitro* oxidation of SOD1 produces aggregates that have the same morphological and structural features as those found in inclusion bodies in ALS. The presence *in vivo* of misfolded mutant SOD1 characterized by alterations in native disulfide bonds and being prone to oligomerization has been recently confirmed.
The intracellular factors that modulate folding and stability of SOD1 are largely unknown but a role in reducing the SOD1 disulfide and destabilizing ALS mutants has been reported for the major cytosolic Glutaredoxin of yeast. The thiol disulfide oxidoreductases glutaredoxins specifically and efficiently reduce mixed disulfides to protein thiols, maintaining thiol homeostasis both in the cytosol and mitochondria. Moreover, both glutaredoxin 1 (cytosolic) and 2 (mitochondrial) are known to prevent mitochondrial disfunction due to several neurotoxic stimuli.

Methods: A human neuroblastoma cell line (SH-SY5Y) and murine motoneuronal cell line (NSC-34) have been engineered for the over-expression of glutaredoxins either in the cytoplasm (Grx1) or in mitochondria (Grx2). In these cell lines, the expression of a wide panel of mutant SOD1s have been induced by adenoviral vectors. These lines have been used to characterize the biochemical properties of mutant SOD1s by utilizing non reducing SDS-PAGE on protein extracts from different cell fractions. Moreover, spectrophotometric and immunochemical assays have been performed in order to measure cell viability, mitochondrial functionality and apoptotic activation.

Results: Mitochondrial Grx2 protects neuroblastoma cell lines from apoptotic insult induced by mutant SOD1 by inhibiting caspase-3 activation and by interfering with mitochondrial fragmentation observed in cell lines expressing mutant SOD1s. Moreover, cell lines expressing mutant SOD1 show a dramatic alteration of the mitochondrial network, which is reverted by overexpression of Grx2. In line with this observation, preliminary data indicates that Grx2 modifies the expression patterns of proteins which play a key role in mitochondrial dynamics, such as Drp-1 and OPA1. On the other hand, cytosolic Grx1 increases solubility of mutant SOD1 in the cytosol but does not inhibit apoptosis in cell lines expressing mutant SOD1.

Discussion and Conclusion: These data confirm the central role of mitochondrial damage in ALS and contribute to clarify the role of mutant SOD1 oligomerization/aggregation in the cascade of events that trigger the death of motor neurones. In perspective, this study might contribute to the development of strategies for the treatment of ALS.

P76 MOTONEURON SUBTYPES IN DEVELOPMENT AND ALS: A FACS-BASED APPROACH

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Keywords: motoneuron subtype, vulnerability, fluorescence-activated cell sorting/FACS

Motoneurons remarkably differ in morphology, function and vulnerability to neurodegeneration and ALS. The aim of this study is to isolate and study motor neuron subtypes by developing a novel approach based on fluorescence-activated cell sorting (FACS).
Axial and hindlimb motoneurons were isolated from Hb9:GFP embryonic mouse spinal cords and their gene expression profiles, electrical and morphological properties were compared. Limb motor neurons had a high whole cell capacitance indicating a large soma; grew long axons with few, mostly terminal branches; and responded in a dose-dependant manner to HGF (hepatocyte growth factor), a neurotrophic factor produced in limb mesenchyme. In contrast, axial motor neurons had a low whole cell capacitance, grew short axons with numerous proximal branches and were unresponsive to HGF. Forced Hb9 over-expression in limb motor neurons stimulated axon branching but attenuated axon extension indicating a direct role of Hb9 in both processes. The distinct morphologies of limb and axial motor neurons thus reflect their respective roles in fine versus bulk movement and their presumptive differences in motor unit size.
As a second example, the proteomic profiles of FACS-isolated lumbar motor neurons from control mice were compared with SOD1 G93A mice. 2D-gel analysis of protein extracts revealed the presence of more than 500 protein spots. Eight proteins were found to be dysregulated by mutant SOD1 and these are currently being analyzed.
In conclusion, FACS-based approaches hold great potential for analyzing normal development and ALS-linked vulnerability of motoneuron subtypes.

P77 RETINOIC ACID RECEPTOR-MEDIATED SIGNALING IN MOTOR NEURON-ENRICHED CULTURES: IMPLICATIONS FOR ALS

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Keywords: gene expression, nuclear receptors, retinoic acid

Background: Although the mechanisms underlying the motor neuron degeneration that occurs in ALS are poorly understood, recent work indicates that altered retinoid signaling may contribute to the disease process. This pathway impacts gene expression as the nuclear receptors for retinoic acid function as ligand-activated transcription factors. The retinoic acid receptors (RARs) are activated by the predominant retinoic acid isoform (all-trans retinoic acid: ATRA) and mediate gene expression by forming heterodimers with the retinoid X receptors (RXRs). Alterations in specific RAR isotypes have recently been observed in post-mortem tissue from ALS patients when compared to control subjects. To further define the role of the retinoid signaling pathway in motor neuron cell death, additional model systems that allow the direct modulation of the retinoid pathway in motor neurons must be developed.

Objectives: To investigate the effects of RAR-mediated signaling on motor neuron survival and morphology. To characterize the downstream targets of the RARs in an isotype-specific manner.

Methods: Motor neuron-enriched cultures were established from embryonic day 14 rats and used to investigate the role of the retinoid signaling pathway. Pan-agonists (including ATRA) and antagonists as well as isotype-specific agents were used to modulate RAR-mediated signaling. In addition, toxicity studies using hydrogen peroxide as a model of oxidative stress/injury were performed in the presence and absence of these agonists and antagonists.

Results: In the presence of ATRA, the number of branch points/cell was decreased in motor neurons. RARα exhibited a diffuse cytoplasmic pattern while RARβ was localized to the nucleus irrespective of ATRA presence or concentration. When ATRA was administered in the presence of hydrogen peroxide, motor neuron cell death was delayed.

Discussion and Conclusions: An increased nuclear RARβ has been observed in patients with sporadic ALS and generated a motor neuron-enriched cell culture system to characterize the transcriptional impact of RARβ in motor neurons. These results indicate that signaling through the RARs maintains long neurites with less branching in motor neuron-enriched cultures. Stimulating the RARs with ATRA significantly delays hydrogen peroxide-induced cell death. Agonists to specific RAR isotypes can replicate this neuroprotective effect. Downstream genes modulated by RARs are currently being examined using microarray analyses.

P78 ANGIOGENIN SIGNALLING IN THE SPINAL CORD

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Background: Amyotrophic Lateral Sclerosis (ALS) is a late onset and fatal neurodegenerative disease of the motor system. Approximately 10% of ALS cases are dominantly inherited whereas 90% of patients exhibit highly similar clinical presentation but sporadic onset of the disease, thereby suggesting a varied root cause. It is also likely that a circuitous mechanism contributes to neuronal loss in ALS since the interaction between motoneurons and astroglia can markedly affect neuronal viability and disease progression. Although mutations in the Superoxide Dismutase 1 (SOD1) locus represent the most common genetic determinant, coding mutations in angiogenin, a hypoxia-inducible factor, which segregate within ALS pedigrees have also been identified. Moreover, the intraperitoneal administration of angiogenin has been shown to slow disease progression and extend the life span of SOD1 transgenic mice. Angiogenin shows potent neuroprotective properties *in vitro* and *in vivo*.

Objectives: To study the mechanism of motoneuron protection mediated by treatment with recombinant angiogenin and to extrapolate the role of endogenous angiogenin with respect to this function.

Methods: Primary murine spinal cord co-cultures were used to study angiogenin uptake and distribution. Angiogenin was visualised using fluorescence and confocal laser microscopy in conjunction with fluorescein-tagged recombinant human angiogenin or immunocytochemistry with antibodies against human or mouse angiogenin. Neuroprotection was assessed in co-cultures using a motoneuron-specific antibody in parallel with MTT viability assays. Mouse cortical astrocyte cultures were used to study the mechanism of angiogenin internalisation and astrocyte-specific signalling, which was analysed by Western blotting. The NSC-34 motoneuron-like cell line was used to biochemically assay for angiogenin secretion.

Results: Angiogenin is expressed and enriched in motoneurons, however application of recombinant human angiogenin to mixed spinal cord cultures revealed an exclusively non-neuronal uptake of the protein. Internalisation is inhibited by ATP-depletion, actin depolymerisation, high sodium, monodansylcadaverine and angiogenin denaturation, suggesting a proteoglycan-assisted clathrin-dependent mechanism. After internalisation, angiogenin remains sequestered in non-acidic cytosolic vesicles, yet causes increased nrf2 protein expression and glutathione accumulation. A paracrine mechanism of neuroprotection was tested and showed that medium conditioned by astrocytes treated with angiogenin can stimulate Akt phosphorylation in motoneuron cultures and protect motoneurons from AMPA-mediated toxicity. There is evidence to indicate that motoneurons upregulate and secrete angiogenin under stress.

Discussion and Conclusions: This data suggests that a model in which motoneurons utilise angiogenin to initiate a pro-survival signalling circuit through neighbouring glia. These findings also help clarify the mechanism by which angiogenin administration mediates neuroprotection *in vivo*.
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P79 STEROL REGULATORY BINDING PROTEIN-1 (SREBP1) IN ALS: A TARGET FOR THERAPEUTIC INTERVENTION

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Keywords: sterol regulatory binding protein-1, excitotoxicity, lipid homeostasis

Background: ALS (amyotrophic lateral sclerosis) is the most common adult onset Motor Neuron Disease (MND), with an incidence of ~2.1 per 100,000 persons/year. Excitotoxicity or excessive activation of glutamate receptors has been implicated in a variety of neuropathological conditions including ALS. It has recently been demonstrated that, in primary cortical neuronal cultures and in the rat transient middle cerebral artery occlusion (MCAo) model of focal ischemic stroke, excitotoxic neuronal death is associated with a NMDAR- and time-dependent activation of SREBP-1. SREBP-1 is a transcription factor that regulates the expression of genes involved in maintaining lipid homeostasis. Degradation of Insig-1, an endoplasmic reticulum (ER) membrane resident protein, is a prerequisite for SREBP-1 activation. This study established a direct linear correlation between excitotoxicity-induced neuronal cell death and SREBP-1 activation. Indip, a TAT fused peptide which blocked SREBP1 activation by stabilizing Insig-1, was found to efficiently protect neurons against excitotoxicity-induced death.

Objectives: To study Insig-1 degradation and SREBP-1 activation in the spinal cord of transgenic G93A mouse model of ALS, as well as post-mortem specimen of human familial (FALS) and sporadic (SALS) ALS. To investigate neuroprotective effects of Indip against excitotoxicity in both wild type and transgenic (tg) cultured spinal cord neurons.

Materials and Methods: We used immunoblotting to investigate Insig-1 degradation and SREBP-1 activation. Both LDH and TUNEL assays were used to study neuroprotective effects of Indip (compared with a control peptide).

Results: In spinal cords of transgenic G93A model of ALS and post-mortem specimens of human ALS (FALS and SALS) Insig-1 was drastically degraded, likely leading to significant SREBP-1 activation. Indip (2 μM) significantly protected wild type mouse cultured spinal cord neurons against glutamate (100 μM, 30 min) induced cell death, while control peptide failed to exert any protective effects (LDH assay). Similar results were obtained with cultured G93A spinal cord neurons using TUNEL assay.

Discussion: Lipid metabolism has been recently implicated in the pathogenesis of ALS. This study identifies activation of SREBP1 as a novel step in the signaling cascades leading to excitotoxic neuronal death in ALS.

Conclusion: These results suggest that agents that reduce SREBP1 activation, such as Tat-Indip, may represent a new class of neuroprotective therapeutics against ALS. Studies of therapeutic manipulation of this pathway in G93A mice are underway and will be reported at the Symposium.

P80 SULFORAPHANE PROTECTS MOTOR NEURONS OF RAT SPINAL CORD VIA INDUCING PHASE II ENZYMES

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Keywords: glutamate excitotoxicity, phase II enzyme, sulforaphane

Background: Amyotrophic lateral sclerosis is a lethal neurodegenerative disease, characterized by degeneration of motor neurons from the cortex, brainstem and spinal cord. Present evidence indicates that motor neuron loss results from a complex interplay among oxidative injury, excitotoxic stimulation, aggregation and/or dysfunction of critical proteins as well as genetic factors. Recent investigations support that these mechanisms are not mutually exclusive but are activated as a communal response that may be coordinated by oxidative stress. It is reported that the activation of the Nrf2/ARE signaling pathway could induce a series of phase II antioxidant enzymes, which play an important role in cellular defense against oxidative stress.

Objectives: To investigate whether sulforaphane (SF) can protect motor neurons via up-regulating the expression of phase II enzymes.

Methods: Organotypic spinal cord cultures were prepared from lumbar spinal cord explants of 7-day-old SOD1 rats. The cultures were divided into three groups at random: control, Threohydroxyaspartate (THA), SF and THA (SF pretreated for 48 hours then treated with SF and THA). The number of motor neurons was assessed by immunohistochemistry, and the expression of phase II enzymes were assayed with a Western blot.

Results: After 3 weeks treatment, the motor neuron number in the 100 μM THA group decreased significantly compared with the control group ($P<0.05$), while the motor neuron number in 10 μM SF and 100 μM THA group increased significantly as compared with the THA group ($P<0.05$). SF also significantly up-regulated the expression of phase II enzymes including NQO-1 and HO-1 in SF and THA group. However, SF had no effect on the glutamate concentration in the culture media.

Discussion and Conclusions: THA causes an accumulation of synaptic glutamate and over stimulation of the postsynaptic receptor by inhibiting glutamate uptake. THA-induced glutamate excitotoxicity in organotypic spinal cord culture is a well-known model of motor neuron degeneration. Glutamate induced excitotoxicity and oxidative damage are believed to play an important role in the development of a number of central nervous system disorders. These results indicate that SF can protect motor neurons from THA induced exitotoxicity by activating the Nrf2/ARE signaling pathway and up-regulating a series of phase II antioxidant enzymes.
These data identified SF as a promising neuroprotective agent and suggested that induction of phase II enzymes may be an important strategy for protection against glutamate induced excitotoxicity.

P81 CURCUMIN ACTIVATES NRF2-ARE PATHWAY AND PROTECTS ASTROCYTES FROM HYDROGEN PEROXIDE INDUCED OXIDATIVE DAMAGE

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Keywords: Nrf2, curcumin, oxidative stress

Background: Oxidative stress, generally resulting from an imbalance between formation of ROS and limited antioxidant defenses, is considered to play a critical role in neurodegenerative diseases. A promising candidate to reduce oxidative damage is the activation of endogenous antioxidant proteins and enzymes, whose transcription is controlled by Nuclear factor erythroid 2-related factor (Nrf2). Nrf2 plays a central role in the regulation of cellular redox status. Nrf2 has been identified as key transcriptional factor that activates the antioxidant response element (ARE) located in many phase II detoxification enzyme genes. Curcumin is a diferuloylmethane compound, possessing anti-inflammatory activity, antioxidative and tumor suppression properties as shown in many cell types. Recently, it has been reported that curcumin can activivate the HO-1 gene via Nrf2/ARE pathway in renal epithelial cells.

Objectives: To test if curcumin can protect astrocytes against hydrogen peroxide (H_2O_2)-induced oxidative damage by activating Nrf2-ARE pathway and inducing the expression of phase II enzymes.

Methods: Primary astrocytes purified from spinal cord of newborn mice were cultured. ROS production was detected using compound DCF-DA. Cell viability was measured by MTT. MDA content was tested. Western Blot, RT-PCR and confocal microscopy analyses were used. Mitochondria transmembrane potential ($\Delta\Psi m$) was measured by flow cytometry.

Results: H_2O_2 treatment increased ROS and MDA production, mitochondrial damage and decreased cell viability in both wild-type (Nrf2+/+) and Nrf2 knockout (Nrf2 −/−) astrocytes. These effects were more obvious in Nrf2 −/− astrocytes, while pretreatment with 10 μM curcumin led to significant cytoprotection in Nrf2+/+ astrocytes. In contrast, no significant change was observed in Nrf2 −/− cells.
Curcumin caused a significant increase in nuclear Nrf2 translocation and up-regulated Nrf2 target genes in Nrf2+/+ astrocytes while it was abolished in Nrf2 −/− cells.
Exposure to H_2O_2 resulted in substantial loss of phase II detoxification enzymes of HO-1, NQO1 and GCLc. Pretreatment with curcumin significantly increased the expression of these enzymes. In contrast, no significant change was seen in Nrf2 −/− astrocytes. To gain further insight into the protective activity of curcumin, the mitochondrial function integrity was measured and H_2O_2 treatment was found to cause a loss of $\triangle\Psi m$ in Nrf2+/+ astrocytes, which was more obvious in Nrf2 −/− astrocytes. Pretreatment with curcumin efficiently prevented loss of $\Delta\Psi m$ in Nrf2+/+ astrocytes, but not in Nrf2 −/− astrocytes.

Discussion: Oxidative damage has been implicated in the pathogenesis of neurodegenerative disorders. Nrf2 is crucial in controlling the susceptibility and tolerance to oxidative stress. Evidence has shown that activation of the Nrf2-ARE pathway in astrocytes protect neurons from oxidative damage. Curcumin might be a potential candidate for neurodegenerative therapy by activating the Nrf2-ARE pathway.

Conclusions: Curcumin decreases the susceptibility and increases the tolerance to H_2O_2 induced oxidative stress in astrocytes by activating the Nrf2-ARE pathway, decreasing oxidative damage and protecting mitochondria.

P82 IDENTIFICATION OF NOVEL ANTI-OXIDATIVE NEUROPROTECTANTS BY THE NAIP-BASED DRUG SCREENING

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Keywords: NAIP, oxidative stress, neuroprotection

Background: Several lines of evidence have indicated that oxidative stress plays a crucial role in the pathogenesis of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS)/motor neuron diseases (MND). Neuronal apoptosis inhibitory protein (NAIP/BIRC1), a founding member of the inhibitor of apoptosis protein family, is known to protect against oxidative stress-induced cell death. Recently, it has been shown that L-745,870, a potent NAIP-upregulating compound, selectively inhibits oxidative stress-induced cell death *in vitro*, attenuates ischemia-induced neural cell damage in gerbils and suppresses disease progression in an ALS mouse model (1). These studies show a preclinical proof-of-concept for the NAIP-mediated anti-oxidative stress remedy in the treatment of neurodegenerative diseases.

Objectives: To identify the compounds that selectively suppress oxidative stress-induced cell death by adopting the NAIP-based drug screening in conjunction with an *in silico* drug-like screening and to investigate the *in vivo* efficacy of the compound using an ALS mouse model.

Methods: NAIP-based drug screening was used in conjunction with an *in silico* drug-like screening for the identification of drug candidates (2). The identified compounds were evaluated by *in vitro* cell viability assay, RT-PCR, and Western blotting to confirm their protective competence against

oxidative stress-induced cell death. A pre-clinical animal study was also conducted in which a daily oral administration of drug candidates was fed to an ALS mouse model (SOD1-H46R) after signs of disease onset.

Results: Several hit compounds have been identified by the drug screening. Among the compounds, CPN-9 revealed the highest-score for the protection of non-neuronal HeLa cells against the oxidative stress-induced cell death. CPN-9 also selectively protected the differentiated human neuroblastoma SH-SY5Y cells from oxidative stress-induced cell death. CPN-9 treatment resulted in an enhancement of the heme oxygenase-1 (HO-1) gene expression in parallel with an increase in the levels of the HO-1 protein, an anti-oxidant enzyme, in differentiated SH-SY5Y cells. Further to this, the post-onset administration of CPN-9 to ALS mice (SOD1-H46R) slowed the disease progression and prolonged survival interval of CPN9-treated mice compared with vehicle-treated mice.

Conclusion: In this study, CPN-9 selectively protected the differentiated SH-SY5Y cells from oxidative stress-induced cell death. CPN-9 exerts neuroprotective effects, in part, by reducing oxidative stress via the enhanced HO-1 expression. The post-onset administration of CPN-9 to ALS mice (SOD1-H46R) prolonged the survival interval. Collectively, a newly identified compound, CPN-9, may represent a potential neuroprotective drug candidate for the therapeutic treatment of ALS/MND and other neurodegenerative diseases.

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P83 A STUDY ON THE EFFECTS OF P66SHC ON OXIDATIVE STRESS AND CELL DEATH INDUCED BY MUTANT SOD1

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Keywords: mutant SOD1, oxidative stress, p66Shc

Background: Increased oxidative stress and mitochondrial damage are among the mechanisms whereby mutant SOD1 might induce motoneuronal death. Recently, a novel signaling mechanism involving the 66-kilodalton isoform of the growth factor adapter Shc (p66Shc), that is operative in the pathophysiological condition of oxidative stress, has been identified. p66Shc is an alternatively spliced isoform of a growth factor adapter that is phosphorylated upon oxidative stress. In this form, a fraction of p66Shc localizes to mitochondria, where it binds to cytochrome c and acts as an oxidoreductase, generating reactive oxygen species (ROS) and leading to organelle dysfunction and cell death. p66Shc$^{-/-}$ mice exhibit a 30% extended lifespan, reduced H_2O_2 levels and an enhanced resistance against oxidative stress, indicating that p66Shc controls cellular stress responses and mammalian lifespan.

Objectives: To investigate the role of p66Shc in mutant SOD1 (mutSOD1)-induced cell toxicity given the pivotal role of p66Shc in the control of mitochondria-dependent oxidative balance and energetic metabolism.

Methods: Mouse motoneuronal NSC34 cells stably expressing wild-type (wt) or G93A mutSOD1 under the control of a Tet-responsive element and human SH-SY5Y neuroblastoma cells infected with adenoviral vectors coding for wt or G93A mutSOD1, have been used as cellular models of ALS. Genetic manipulation of the p66Shc-dependent molecular pathway has been obtained by the expression of p66Shc proteins, both wild-type and mutant. In particular, an S36A non-phosphorylatable mutant and a double mutant at position 132 and 133 (EEQQ), the site where the redox activity of p66Shc has been mapped, have been chosen because of their ability to inhibit the pro-oxidant, pro-apoptotic function of endogenous p66Shc. Expression of constitutive active (V12) or inactive (N17) Rac1 was achieved by adenoviral vectors.

Results: Upon stress, p66Shc is phosphorylated on serine 36 and this event is crucial to p66Shc-mediated oxidative stress and apoptosis. To investigate the role of p66Shc in mutSOD1-induced cell stress, NSC34 cells were analyzed by Western blotting using an antibody that specifically recognized phosphorylated p66Shc at Ser36. Expression of G93A mutSOD1, but not wtSOD1, induced Ser36-p66Shc phosphorylation. Similar results were obtained with SH-SY5Y cells infected with adenoviruses expressing mutSOD1. This event is functionally relevant, since the overexpression of an S36A mutant of p66Shc inhibits mutSOD1-induced apoptosis. Similarly, cells overexpressing EEQQ-p66Shc mutant are protected from apoptosis. Interestingly, the apoptotic phenotype induced by mutSOD1 is re-established by the expression of a constitutive inactive form of Rac1, a small GTPase which controls important functions in cells including oxidative metabolism, while a constitutive active Rac1 protects cells from mutSOD1-induced apoptosis.

Discussion and Conclusions: These observations indicate that p66Shc is recruited in the molecular pathways activated by mutSOD1 in cells and that the toxic function of mutSOD1 is mediated by a p66Shc-dependent deregulation of Rac1 activity. The molecular mechanisms regulating this pathways are under investigation.

P84 TUMOR NECROSIS FACTOR ALPHA AND INTERFERON GAMMA COOPERATIVELY INDUCE OXIDATIVE STRESS AND MOTONEURON DEATH IN A NEW MODEL OF RAT SPINAL CORD EMBRYONIC EXPLANTS

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Keywords: microglia, proinflammatory cytokines, oxidative stress

Background: The accumulation of reactive microglia in amyotrophic lateral sclerosis (ALS) tissue creates a chronic inflammatory environment that results in motoneuron death. The proinflammatory cytokines TNF-α and IFN-γ have been proposed to be involved in ALS-linked microglial activation and increased levels of these cytokines have been reported in the blood of ALS patients and in the spinal cord of the transgenic mouse model.

Objectives: To develop a new culture system to assess the interaction between TNF-α and IFN-γ on microglial activation,

nitric oxide and oxidative stress generation and the implications on the viability of spinal cord motoneurons.

Methods: Lumbar spinal cords were dissected from embryos (E15-16) of Sprague-Dawley rats. Cords were transversely sectioned into 350 micron slices and sections placed on 35 mm-wells precoated with poly-DL-ornithine (30 μg/ml) and L-laminin (2 μg/ml). All treatments started 10 days after the explant procedure.
Explants were exposed for 48 hours to IFN-γ (10 ng/ml), TNF-α (10 ng/ml) or both cytokines together, in the presence of absence of the selective iNOS and NADPH oxidase inhibitors, AMT (10 μM) and apocynin (1 mM), respectively.

Results: After 5 days *in vitro*, some cells of the spinal cord started the migration outside the explant. Motoneurons migrated from the ventral side of the spinal cord and were identified through co-labelling with SMI-32, Islet1 and anti-vesicular acetylcholine transporter (VAChT) antibodies. After 10 days *in vitro*, motoneurons appeared to be forming groups growing in monolayers outside the explant. Other migrating cells were identified as interneurons, astrocytes and microglial cells. The combined exposure to TNF-α and IFN-γ resulted in an increased expression of the pro-oxidative enzymes iNOS, gp91phox and COX-2, as compared to each cytokine alone. This effect was related to their cooperation in the activation of the transcription factor NF-κB. TNF-α and IFN-γ also cooperated to promote protein oxidation and nitration, thus increasing the percentage of motoneurons immunoreactive for nitrotyrosine. Apoptotic motoneuron death, measured through annexin V-Cy3 and active caspase-3 immunoreactivities, was also found to be cooperatively induced by TNF-α and IFN-γ and reverted in the presence of AMT or apocynin. Interestingly, these cytokines did not affect the viability of density gradient purified spinal cord motoneurons in the absence of glial cells.

Discussion and Conclusions: This research has developed a new culture system that facilitates the study of spinal cord motoneurons and in which the activacion of microglia with TNF-α and IFN-γ reproduces some of the features of ALS in terms of pro-oxidative enzymes expression, protein oxidation and nitration and apoptotic motoneuron death. It is proposed that TNF-α and IFN-γ have cooperative/complementary roles in inflammation-induced motoneuron death.

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P85 EXACERBATION OF GLUTAMATE-INDUCED MOTONEURON DEATH BY THE PROINFLAMMATORY CYTOKINE TUMOR NECROSIS FACTOR-ALPHA

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Keywords: excitotoxicity, glutamate transporter-1, tumor necrosis factor-alpha

Background: Besides glutamate excitotoxicity, the neuroinflamatory response is emerging as a relevant contributor to motoneuron loss in ALS. It has been shown that there are high levels of circulating proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), both in human patients and in animal models of ALS. A facilitatory role of TNF-α in glutamate-excitotoxicity, inducing an increase in the surface expression of AMPA receptors and a reduction in the expression of the astroglial glutamate transporter GLT-1, thus impairing glutamate uptake, has been demonstrated in hippocampal neurons. However, the role of proinflammatory cytokines on motoneuron excitotoxicity has not been studied in the context of ALS.

Objectives: To study the effects of the proinflammatory cytokine TNF-α on excitotoxic motoneuron death and the intracellular pathways implicated.

Methods: Organotypic cultures of rat spinal cord were exposed to the glutamate-uptake inhibitor threohydroxyaspartate (THA, 100 μM, 1–4 weeks) as a model of chronic glutamate excitotoxicity that mimics the slow excitotoxicity that occurs in ALS. TNF-α (20 ng/ml) was administered to the cultures, alone or in combination with THA and/or the AMPA receptor antagonist CNQX. The NF-κB inhibitor CAPE was used in some experiments.

Results: Chronic glutamate excitotoxicity resulted in motoneuron loss after 3 weeks. Motoneuron death was associated to a neuroinflammatory response (microglial activation and TNF-α release, and increased expression of COX-2). Exposure to TNF-α for 2 weeks alone did not affect motoneuron survival whereas co-administration of TNF-α and THA, potentiated the excitotoxic motoneuron death, which could be blocked by CNQX. Co-exposure to TNF-α and THA resulted in down-regulation of GLT-1, together with increased glutamate levels in the culture. However, the expression of the AMPA receptor subunits GluR1 and GluR2 was not modified by any of the treatments. TNF-α and THA cooperatively activated the transcription factor NF-κB. The combined exposure to TNF-α and THA resulted in an increased expression of the pro-oxidative enzymes COX-2 and NADPH oxidase along with an increased protein oxidation. The NF-κB inhibitor CAPE abrogated the exacerbation of glutamate-mediated motoneuron death induced by TNF-α, preventing GLT-1 down-regulation and the extracellular accumulation of glutamate and also reducing the levels of oxidative stress.

Discussion and Conclusion: Chronic glutamate excitotoxicity induces a neuroinflammatory response in spinal cord organotypic cultures. TNF-α potentiates glutamate-induced excitotoxicity in rat spinal cord motoneurons and this effect could be related to a NF-κB mediated down-regulation of GLT-1, thus resulting in excessive extracellular glutamate levels. In addition, TNF-α seems to potentiate the oxidative stress caused by the excessive stimulation of glutamate receptors in a mechanism involving the NF-κB pathway. Together, these results indicate a facilitatory role of TNF-α on excitotoxic motoneuron death.

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P86 VEGF PROTECTS MOTOR NEURONS AGAINST EXCITOTOXICITY BY UPREGULATION OF GLUR2

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Background: Influx of calcium ions through the a-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors

is toxic to neurons and contributes to the motor neuron degeneration observed in amyotrophic lateral sclerosis (ALS). The calcium permeability of the AMPA receptor depends on its subunit composition. If the GluR2 subunit is present in the receptor complex, the AMPA receptor is impermeable to calcium.

Objectives: To identify factors up regulating the GluR2 subunit in motor neurons. From previous studies it is known that astrocytes are capable of inducing the GluR2 subunit. One of the growth factors released by astrocytes and involved in the pathogenesis of ALS is vascular endothelial growth factor A (VEGF). Therefore, this study aimed to link these two mechanisms involved in motor neuron degeneration.

Results: In this study, VEGF was identified as a GluR2 inducing molecule. Cultured rat motor neurons pretreated with VEGF displayed higher GluR2 levels. This resulted in AMPA receptor currents with a low sensitivity to the polyamine, 1-naphthyl acetyl spermine, a high rectification index and a low relative calcium permeability, all functional parameters linked to high GluR2 levels. The induction by VEGF of GluR2-containing, calcium-impermeable AMPA receptor on motor neurons rendered them less vulnerable to AMPA receptor mediated excitotoxicity. This effect of VEGF was mediated through the VEGFR2 present on the motor neurons and was due to stimulation of GluR2 at the transcriptional level. Intracerebroventricular treatment with VEGF similarly induced GluR2 expression in the ventral spinal cord of rats and this mechanism could contribute to the protective effect of VEGF on motor neurons.

P87 BRANCHED-CHAIN AMINO ACIDS INDUCE HYPEREXCITABILITY IN CORTICAL NEURONS. ARE THEY INVOLVED IN THE AMYOTROPHIC LATERAL SCLEROSIS ETIOPATHOGENESIS?

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Keywords: branched-chain amino acids, cortical neurons, electrophysiology

Background: Many published epidemiological studies have reported an increased occurrence of Amyotrophic Lateral Sclerosis (ALS) among Italian soccer players. The relationship between ALS and Italian soccer players is still to be elucidated. Some of the hypotheses of ALS risk that have been proposed are the use of toxic substances taken to improve athletic performance and the use of therapeutic drugs employed in excessive doses. In particular, branched-chain amino acid (BCAAs, leucine, isoleucine and valine) supplements are frequently used among athletes to stimulate muscular protein synthesis, to improve both mental and physical performance and to accelerate the body's recovery after particularly intense sport activities. They are widely metabolised in the body and easily taken up by the brain via the L-system transporters of the capillary endothelial cells.

Objectives: To investigate the possible role of BCAAs on the electrophysiological properties of cortical neurons and to compare these treated neurons with those obtained from the G93A ALS mouse model.

Methods: Cortices were removed from mouse embryos and grown in dissociated cell culture. For treated neurons, BCAAs (200 μM) were added to the medium for 2, 4 and 6 days. Neurons were recorded at 8 days old using the whole cell configuration of the patch-clamp technique.

Results: Current clamp electrophysiological recordings from cortical neurons were analysed. In all neurons of control ($n = 24$), in those exposed for 2 days ($n = 11$), 4 days ($n = 13$) and 6 days ($n = 16$) to BCAAs, the membrane passive properties were not significantly different. The injection of $> +60$ pA elicited multiple action potential in all neurons and the inverse of the first interspike interval was taken as an estimate of the cell firing frequency. Control neurons were compared with neurons that had been exposed to BCAAs for 2, 4 and 6 days. Neurons exposed to BCAAs for 4 or more days presented a significantly higher frequency compared to controls ($P < 0.05$). The neuronal hyperexcitability was also induced by the single branched -chain amino acid treatment (200 μM) and this effect was specific to these amino acids because cortical neurons exposed to alanine or phenylalanine presented excitability properties comparable to those of control. Interestingly, the frequency values related to control neurons were comparable with previous published values and those related to 6 days exposure to BCAAs were comparable to values related to G93A cortical neurons (1).

Conclusions: These data show that in cultured cortical neurons BCAAs induce hyperexcitability which is a property of G93A neurons, indicating for the first time a possible involvement of BCAAs in ALS etiopathogenesis.

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P88 DISRUPTIONS OF [CA]I AND MITOCHONDRIA IN THE ADULT SOD1 G93A MOUSE MODEL OF ALS AS REVEALED BY RAPID CCD IMAGING OF CNS SLICE PREPARATIONS

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Keywords: CCD imaging, slice preparations, metabolic signature

Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of motoneurons (MNs) in the brain stem and spinal cord. While disruptions of intracellular calcium concentration ([Ca]i) and mitochondria have been associated with selective MN degeneration, the underlying mechanisms are little understood. In this report, simultaneous patch clamp recordings and rapid CCD imaging from slice CNS preparations were utilized to evaluate [Ca]i and metabolic signatures in MNs of young and adult SOD1 G93A animals (B6SJL mice; P90–P120).

At first, we compared the [Ca]i/mitochondrial system in selectively vulnerable and resistant motoneuron types in young wild – type (wt) mice. In vulnerable hypoglossal motoneurons (HMNs), disruption of the mitochondrial electrochemical potential by bath application of the mitochondrial "uncoupler" FCCP provoked a significant retardation of cytosolic Ca^{2+} clearance rates and substantial Ca^{2+}

release events from mitochondria-controlled stores in somatic and dendritic compartments. These observations were different from those obtained in selectively resistant dorsal vagal (DVN) and oculomotor neurons (OMN) under identical experimental conditions. Both DVN and OMN displayed only minor Ca^{2+} release events after FCCP application, where peak amplitudes were approximately 4 fold smaller compared to those in HMNs. Moreover, FCCP did not significantly affect cytosolic Ca^{2+} clearance rates in DVN and OMNs.
[Ca]i and metabolic signatures were further investigated in slice preparations from adult WT and SOD1G93A animals (P90–P120) by using fluorescence dyes (Rhod123, Fura-AM) and intrinsic NADH fluorescence. In MNs and glial cells of the brain stem, NADH fluorescence changes after inhibition of mitochondrial complex IV indicated a disturbed respiratory chain in SOD1G93A animals. Furthermore, measurements based on Rhod123 suggested a severe disruption of the mitochondrial electrochemical potential. A third series of experiments utilizing Fura-AM investigated the Ca/mitochondrial interplay in SOD1 G93A compared to WT animals. Taken together, these results suggest that SOD1 G93A animals are characterized by a substantial disruption of the [Ca]i/mitochondrial system in vulnerable motoneuron populations. These obseravtions are in good agreement with models suggesting that impairments of [Ca]i regulation and mitochondria are critical elements of ALS pathogenesis.

P89 DIFFERENCES BETWEEN BRAIN AND SPINAL CORD IN TOTAL CALCIUM CONTENT AND MITOCHONDRIAL METABOLISM UNDERLIE HIGHER VULNERABILITY OF THE SPINAL CORD IN ALS

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Keywords: mitochondrial metabolism, calcium, ROS

Background: Mitochondrial dysfunction contributes to the loss of motor neurons in ALS. Calcium-induced apoptosis may be responsible for the loss of motor neurons (1). However, the exact mechanisms that cause selective vulnerability of spinal cord in ALS remain unclear.

Objectives: To answer the question: what mitochondrial features make the spinal cord particularly vulnerable in ALS?

Methods: Brain mitochondria (BM) and spinal cord mitochondria (SCM) were isolated from wild type and G93A SOD1 transgenic rats. Pyruvate, glutamate, malate, succinate and their mixtures were used as substrates. The calcium retention capacities (CRC) were measured in the presence of glutamate, pyruvate, malate, oligomycin and ADP. ROS generation was measured by the Amplex Red method.

Results: In comparison with wild type (WT)-BM, the WT-SCM exhibited: 1. lower respiratory activity with all substrates, except succinate; 2. the state 4 oxidation of glutamate, pyruvate and malate was several times higher; 3. lower (40–50%) content of respiratory complexes and porin; 4. lower CRC (914 ± 54 nmol Ca^{2+}/mg protein) versus BM (1618 ± 143 nmol Ca^{2+}/mg protein); 5. WT-SCM generated 2–4 times more ROS with pyruvate and malate or pyruvate, glutamate and malate. The total tissue calcium contents in spinal cord and brain were correspondingly 925 ± 100 and 122 ± 6 μg Ca^{2+}/mg wet weight. In SOD1 rats the yield of the BM and SCM diminished by 20% and 50% respectively as compared with the WT rats. In comparison with WT-SCM, the rates of SOD1-SCM respiration with glutamate, pyruvate or succinate were not diminished, but there was a 2 fold increase in the State 4 oxidation of pyruvate, glutamate and malate accompanied by a several fold increase in the rate of ROS generation. The total Ca^{2+} content in the SOD1 spinal cord diminished by 15%, and by 6% in BM as compared to WT rats.

Conclusions: SCM have the same unique metabolic features as BM (2). However, in SCM succinate oxidation was much less sensitive to inhibition by malate and oxaloacetate. In SOD1-BM and -SCM there was a significant increase in specific production and oxidation of succinate and associated ROS generation. These data suggest that spinal cord neurons are more prone to the apoptotic and necrotic cell death associated with oxidative stress and calcium overload.

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P90 TARGETED EXPRESSION OF MUTANT SOD1 IN THE MITOCHONDRIAL INTERMEMBRANE SPACE CAUSES INCREASED TOXICITY AND EVOKES A POTENTIAL STRESS RESPONSE

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Keywords: mitochondria, protein quality control, stress response

Background: Amyotrophic lateral sclerosis (ALS) is a debilitating neuromuscular disease characterized by the relentless loss of motor neurons in the brain and spinal cord. 20% of familial cases of ALS are caused by mutations in the Cu, Zn-superoxide dismutase (SOD1), a ubiquitously expressed, free radical scavenging enzyme dually localized in the cytosol and the mitochondrial intermembrane space (IMS). In mouse models of ALS, mitochondrial abnormalities are present at the presymptomatic stages; beginning with mitochondrial swelling and vacuolization and eventually leading to motor neuron cell death via caspase activation. Gradual failure in calcium handling and electron transport are also observed. Despite our knowledge of these events, the precise mechanisms that result in specific impairment of spinal cord mitochondria by mutant SOD1 remain to be understood.

Objectives: To determine the degree of toxicity associated with expressing mutant SOD1 in the IMS and identify key regulators of protein homeostasis in this compartment.

Methods: Constructs have been created that target wildtype or mutant (G85R and G93A) hSOD1 to the mitochondrial intermembrane space. NSC-34 cells were transfected with IMS targeting constructs and cell survival was assessed by MTT assays. Changes in gene expression of pathways that

may be activated in response to mutant SOD1 in the IMS were investigated by qPCR.

Results: This study showed that the toxicity of mutant SOD1 is exacerbated when expressed in the IMS compared to the cytoplasm and this toxicity is only associated with motor neuron-like cells. Expression of mutant SOD1 in NSC34 and HEK293 cells results in the upregulation of a number of genes involved in protein folding and degradation within the IMS.

Conclusions: This study successfully targeted wildtype and mutant SOD1 to the mitochondrial intermembrane space. Expression of mutant SOD1 in this compartment induces cell death specific to motor neuron-like cell lines and also leads to changes in gene expression of components involved in protein import and quality control in the IMS. Whether these gene expression changes represent an IMS-specific stress response pathway evoked by SOD1 misfolding remains to be determined.

P91 MUTATED G93A SOD1 DISTURBS THE ER-MITOCHONDRIA-CALCIUM CYCLE IN NSC34 CELLS

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Keywords: ER, mitochondria, calcium

Background: Mutated superoxide-dismutase 1 (SOD1) causing familial amyotrophic lateral sclerosis (ALS) impairs mitochondrial calcium uptake, increases reactive oxygen species (ROS) production and alters the expression of electron transport proteins in mitochondria. Ongoing excitotoxicity, mitochondrial dysfunction and protein misfolding are thought to derail the endoplasmic reticulum (ER) mitochondria calcium cycle (ERMCC) whose role in both sporadic and familial ALS is poorly understood. Recently, real-time monitoring of the ERMCC has become available on a molecular level in cell models of ALS using combined FURA-2/FRET imaging in NSC34 cells transfected with organelle-targeted fluorescent calcium indicators.

Objectives: To determine the effect of expressing human mutated G93A SOD1 on the function of the ERMCC in NSC34 cells.

Methods: Wild-type SOD1, G93A SOD1 and control NSC34 cells were transfected with cDNA of the ER targeted calcium sensitive CFP/YFP cameleon indicator D1ER using lipofectamine(r) at 1 μg/ml. FURA-2 calcium imaging and D1ER CFP/YFP FRET reported the relative calcium concentration in the cytosol ($[Ca^{2+}]_c$) and the ER simultaneously. Fluorescence ratios were calculated online by custom scripting for switching emission filters and excitation wavelengths on an Apple MacIntosh workstation with images grabbed from a hamamatsu orca CCD camera.

Results: Transfection of D1ER cDNA succeeded in 5–10% of control NSC34, 3–5% of wild type NSC34, and 1–2% of mutated SOD1 NSC34 cells. FURA-2 AM was taken up in all cells. Application of caffeine (20 mM) for 1 minute resulted in a rapid increase in $[Ca^{2+}]_c$ from ~100 nM to 0.5–1.0 μM, decayed to plateau and returned to baseline within seconds after caffeine removal. Calcium concentration in the ER ($[Ca^{2+}]_{ER}$) decreased continuously in the presence of caffeine and afterwards returned to baseline within minutes following the kinetics of the ERMCC as previously described (1). In the presence of wild-type SOD1, the kinetics of the ERMCC became unstable with increased variation in peak rise and decay constants in $[Ca^{2+}]_c$ and recovery constants in $[Ca^{2+}]_{ER}$ without statistically significant changes compared to control NSC34. In the presence of G93A mutated SOD1, the decay from the initial calcium peak was slower than both control and in wild-type SOD1 while $[Ca^{2+}]_{ER}$ kinetics reflected a slow recovery from caffeine induced calcium release.

Discussion and Conclusions: Within the ERMCC ER calcium release, passage through the cytosol and calcium release from mitochondria were normal in the presence of G93A mutations in NSC34 cells. Uptake into mitochondria and consequent reuptake into the ER were slower, suggesting G93A mutation induced organelle dysfunction. Further studies using specific blockers of molecular calcium signalling pathways are required to dissect the causal link between the presence of G93A and the induced dysfunction of the ERMCC. Stabilizing the ERMCC pathways may in the future provide neuroprotection for motor neurones in ALS.

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P92 INSIGHTS INTO THE TRAFFICKING OF ASTROCYTIC EAATS – OPTIMIZING CELL-SURFACE EXPRESSION FOR MOTOR NEURON PROTECTION

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Keywords: astrocyte, EAAT2, trafficking

Background: A key role of astrocytes is the removal of the neurotransmitter L-glutamate (Glu) from the extracellular space to preserve normal synaptic signalling and prevent excitotoxic neuronal death. Glu uptake is performed by excitatory amino acid transporters (EAATs), with EAAT2 responsible for the bulk of Glu uptake in the CNS. In models of Motor Neuron Disease (MND), juxtaposed glial cells strongly influence the demise of the motor neurons (MNs) by contributing to inflammatory mechanisms. Increased EAAT2 expression has been shown to be neuroprotective, with EAAT dysfunction in astrocytes playing a primary role in the excitotoxic component of MN injury. Specific pathways regulating the cell-surface expression of EAATs are poorly understood and may provide valuable information about EAATs modulation to treat or prevent MND.

Objectives: To understand the mechanisms that affect the long and short term regulation of EAAT activity in astrocytes by altering astrocytic phenotype and cell-surface expression of EAATs through pharmacological manipulation.

Methods: In this study, primary cultures of mouse astrocytes were treated with rottlerin (a PKCd inhibitor (100 mM, 6 hours (h))) to reduce EAAT activity. Rottlerin was then

removed and the 'recovery' of EAAT activity was investigated. Further, this study attempted to alter the recovery of EAAT activity by blocking synthesis and trafficking using a number of drugs (brefeldin A, nocadazole and monensin) which act on specific intracellular trafficking pathways. Changes in astrocyte morphology and EAAT distribution were determined using fluorescent markers (cytoskeletal markers, markers of intracellular compartments e.g. endoplasmic reticulum) and confocal microscopy.

Results: [^{3}H]D-aspartate uptake was decreased to ~20% of control with rottlerin treatment (100 mM, 6 h) and returned to control levels between 4 ~6 h following rottlerin removal (n = 4). Rottlerin caused rearrangement of the cytoskeleton and redistribution of EAAT expression. Addition of brefeldin (50 mM, 24 h) had no effect on [^{3}H]D-aspartate uptake, but caused a ~50% reduction in cell viability (0.25–6 h). Nocadazole (10 mM, 1 h) had no effect on uptake or cell viability. Monensin (100 mM, 2 h) increased the rate of recovery of [^{3}H]D-aspartate uptake and temporal analyses revealed it was able to increase uptake as early as 0.25 h post-treatment.

Discussion: Pharmacological manipulation of trafficking using brefeldin A, nocadazole and monensin had varied effects. Monensin, causes disruption of microtubules and the trans-Golgi apparatus cisternae as well as altering lysosomal and endosomal function. In endocytosis, monensin can prevent intracellular degradation. This may explain the rapid increase in uptake recovery, with a greater percentage of internalized EAATs available to be re-inserted into the plasma membrane.

Conclusions: This study provides information on the trafficking of EAATs and suggests that optimization of cell-surface expression can be achieved by manipulation of intracellular pathways involved in endocytosis and retrograde transport.

P93 ASTROCYTIC PROTECTION OF SPINAL MOTOR NEURONS BUT NOT CORTICAL NEURONS AGAINST LOSS OF ALS2/ALSIN FUNCTION

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Keywords: astrocyte, non cell autonomous

Three neurodegenerative diseases affecting upper and/or lower motor neurons have been associated with loss of ALS2/Alsin function: juvenile amyotrophic lateral sclerosis, primary lateral sclerosis and infantile-onset ascending hereditary spastic paralysis. The distinct neuronal vulnerability and the role of glia in these diseases remain unclear.
Alsin-depleted spinal motor neurons can be rescued from cell death and defective axon growth by co-cultured astrocytes. The astrocytic rescue is mediated by a soluble protective factor rather than by cellular contact. Cortical neurons are intrinsically as vulnerable to alsin depletion as spinal motor neurons but cannot be rescued by co-cultured astrocytes.
The data highlights non cell-autonomous glial effects in a recessive form of motor neuron disease and provide a potential rationale for the higher vulnerability of upper versus lower motor neurons in ALS2/Alsin-linked disorders. Identifying the molecular nature of the astrocytic protective factor and its intracellular signalling mechanism will help to develop new neuroprotective strategies for these disorders.

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P94 MUTATIONS IN THE GENES ENCODING TDP-43 AND FUS AND THEIR FUNCTIONAL CONSEQUENCES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FUS, TARDBP, immunohistochemisty

Background: ALS (amyotrophic lateral sclerosis) is an adult-onset neurodegenerative disorder that causes degeneration of both upper and lower motor neurons. The principal pathology of ALS is the presence of ubiquitin positive protein aggregates in the cell body of the motor neurons. TDP-43, principally a nuclear protein (encoded by TARDBP gene) is a major component of the ubiquinated inclusions (UBIs) in ALS. TDP-43 pathology is present in almost all ALS (familial and sporadic) cases except SOD1-positive cases. Several TARDBP mutations have recently been reported in both familial and sporadic cases. Another gene which has recently been implicated in the pathogenesis of familial ALS is the FUS gene. Mutations in FUS are the second most common known gene abnormality in familial ALS (FALS) after SOD1. FUS has a functional homology (both are components of ribonucleoprotein complexes) to the TARDBP gene, which suggests that a common mechanism may underlie motor neuron degeneration.

Objectives: To screen for additional mutations in TARDBP and FUS genes among an extended familial ALS cohort (n = 70 for screening TARDBP gene and n = 28 for screening FUS gene). Another aim is to establish neuronal cell models expressing mutant TDP-43 and FUS. The effects of TARDBP mutations will also be investigated in lymphoblasts.

Methods: PCR and sequencing techniques were used to screen the familial ALS cohort. Transfection, cell toxicity assays, cell stressing methods, immunohistochemistry, immunofluorescence and Western blotting approaches were employed to establish neuronal cell models and to the study of patient cells.

Discussion: One new TARDBP gene mutation was found in the extended familial ALS cohort (n = 70). The functional consequences of novel mutation (G294V) and recently published TARDBP mutations (M337V, Q331K, G294A) were analysed in this study. One new FUS mutation R521H was found in the extended FALS cohort and will be further investigated in this study. Preliminary immunohistochemistry and immunofluorescence results show that there is presence of aggregates and an abnormal redistribution of TDP-43 from the nucleus to the cytoplasm in cells tranfected with TARDBP mutations when different cellular stresses are induced. These phenotypic changes under different stresses implicate pathways and mechanisms through which TDP-43 plays a

pathogenic role. Hyperphosphorylation and N-terminal cleavage of TDP-43 are also currently being analysed with Western blots. The effects of induced cellular stresses on patient lymphoblasts are also in progress.

P95 INVESTIGATIONS ON THE P56S VAPB PROTEIN AND EXAMINATION OF ITS ROLE IN THE PATHOGENESIS OF ALS8

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Keywords: fALS, VAPB

A mutation (P56S) in the MSP-domain of the vesicle-associated membrane protein-associated protein B (VAPB/ALS8) is linked to autosomal dominant familial amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy which has thus far only been described in the Brazilian population of Portuguese origin. Resulting clinical phenotypes of motor neuron disorders are heterogeneous and range from late-onset spinal muscle atrophy (SMA) to proximal SMA, atypical ALS and typical ALS disease progression.

This study describes the first case of the P56S mutation in a German patient who presented with a lower motor neuron onset at the age of 41 years with proximal leg muscle weakness and fasciculations, but no upper motor neuron signs. The deceased mother and maternal grandfather had suffered from a similar disease. Haplotype analysis indicated that the mutation was not derived from the same founder as the one in the Brazilian families. Thus, the P56S VAPB mutation has been found in non-Brazilian patients and is not due to a single founder. The pathophysiology underlying ALS8 and the cellular mechanisms of the mutated VAPB proteins leading to motor neuron degeneration are largely unknown. VAPB interacts with proteins involved in the exocytosis of synaptic vesicles. VAPB also seems to play a role in the unfolded protein response and in the transport of ceramide from the ER to the Golgi apparatus.

The above mentioned P56S mutation leads to the formation of protein aggregates when expressed in cell culture models and was shown to disrupt the interaction of VAPB with several proteins including β-Tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This study found that mutated VAPB forms aggregates within transfected cells including the motor neuron cell line NSC-34 whereas wild-type VAPB does not. In cells co-transfected wild-type and mutated VAPB the wild-type protein is recruited into aggregates by mutated VAPB, explaining the dominant mode of inheritance of ALS8. Furthermore, the mutant protein is strongly polyubiquitinylated. Accumulated polyubiquitinylated P56S VAPB might obstruct the proteasomal degradation pathway. Recently, five undescribed alternative transcripts of VAPB in cDNAs were discovered from different human tissues. The role of these transcripts are currently under investigation.

P96 HFE POLYMORPHISMS AND ALS: INSIGHTS INTO A PUTATIVE RISK FACTOR VIA TAU PHOSPHORYLATION MECHANISMS

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Background: Polymorphisms in the HFE gene, specifically the H63D HFE variant, are reportedly increased in individuals with amyotrophic lateral sclerosis (ALS). Cognitive impairment frequently appears in the ALS patient population and HFE polymorphisms have been found to be associated with other neurodegenerative disorders that exhibit cognitive deficits. A recent study (1) found an up-regulation of glycogen synthase kinase-3 beta (GSK-3β) in the frontal and temporal cortex in ALS patients with cognitive impairment.

Objectives: To test the hypothesis that the presence of H63D HFE may lead to an up-regulation of GSK-3β and subsequent increase in tau phosphorylation.

Methods: A stable human neuroblastoma SH-SY5Y cell line was used to study the influence of HFE polymorphisms on mechanisms of tau phosphorylation including the cellular kinase, GSK-3β.

Results: This study shows that expression of H63D HFE is associated with increased tau phosphorylation at the serine 199, serine 202, serine 396 and serine 404 amino acid sites in the presence of the H63D allele. Through evaluating the direct mechanisms responsible for increased tau phosphorylation an increased activity of GSK-3β has been found. Protein phosphatase (PP-1 and PP-2A) expression was not altered under the expression of H63D HFE. The H63D polymorphism-associated changes of GSK-3β activity could be mimicked by changing iron status in the cells expressing wild-type HFE.

Discussion: These studies establish that the H63D HFE genetic variant could be a risk factor for neurodegenerative diseases like ALS by increasing GSK-3β activity resulting in increased tau phosphorylation and subsequent cognitive impairment. Furthermore the data strongly suggest that HFE polymorphisms should be considered when assessing therapeutic interventions in ALS patients displaying cognitive deficits.

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THEME 4 HUMAN CELL BIOLOGY AND PATHOLOGY

P97 GENERATING NEURAL REPORTER LINES IN HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS BY GENE TARGETING

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Keywords: human embryonic stem cells, induced pluripotent stem cells, gene targeting

Background: Human embryonic stem cells (hESCs) and induced pluripotent stem (iPS) cells are invaluable tools to model human diseases *in vitro*. Currently, ALS disease models are largely limited to transgenic rodents.

Objectives: To establish a highly efficient gene targeting protocol in hESCs and iPS cells in order to accelerate the development of ALS disease models using human cells. As a proof of principle, a GFP reporter to Olig2, a basic helix-loop-helix transcription factor important in both motoneuron and glia development, in hESC line BG01 was targeted.

Methods and Results: A GFP cassette was targeted to one allele of the Olig2 locus in BG01 with an efficiency of 5.7%. One of the homologous recombinants was named R-Olig2 and tested further. R-Olig2 (like the other clones) retained typical hESC morphology, the expression of pluripotency and self-renewal markers, the ability to differentiate into all three germ layers and a normal parental karyotype. Most importantly, when R-Olig2 was induced by a sonic hedgehog agonist and retinoic acid, GFP expression recapitulated endogenous Olig2 expression. We were able to harvest and purify GFP+ cells by fluorescence-activated cell sorting (FACS) from different culture stages.

Discussion and Conclusion: Consistent with previous reports from rodent studies, early GFP-expressing cells appeared biased to a neuronal fate while cells harvested from prolonged cultures appeared biased to an oligodendrocytic fate. GFP+ cells harvested from early stages of differentiation were able to generate motoneurons that caused aggregation of acetylcholine receptors when co-cultured with myoblasts, as detected by immunocytochemistry using an antibody against α-Bungarotoxin. GFP+ cells harvested from late stages of the induction culture did not differentiate into motoneurons but were able to differentiate into glial cells that were A2B5 immunoreactive and expressed PDGFRa and NG2. When further differentiated, these cells became GalC+ oligodendrocytes and GFAP+ astrocytes both *in vitro* and *in vivo* after transplantation into adult rat spinal cords. Gene expression profiles of both early and late sorted GFP+ cells were distinct. Early stage GFP+ cells differentially expressed molecules that are important in motoneuron specification and maturation. In contrast, genes that are involved in the commitment of glia and oligodendrocyte differentiation and myelination were upregulated in late stage GFP+ cells. In conclusion, a GFP reporter line was generated by targeting the Olig2 gene using homologous recombination in hESCs. GFP expression faithfully replicates the endogenous Olig2 expression and can be used as a surrogate marker to purify Olig2+ cells which are able to generate motoneurons and glial progenitors. This protocol will be extended to generating ALS disease models in hESCs and reporter lines from patient-specific iPS cells.

P98 THE ROLE OF CONNECTIVE TISSUE PROTEINS IN ALS

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Keywords: fibronectin, plasma

Background: Several morphological and biochemical studies of skin in patients with sporadic amyotrophic lateral sclerosis (ALS) have demonstrated 1) a significant negative correlation between the diameter of collagen fibrils and duration of illness; 2) decreased content of collagen; 3) alteration of cross-linking of collagen; and 4) increased solubility of collagen. Type IV collagen is composed of the major triple-helix, the aminoterminal triple-helix domain (7S domain) and the carboxyterminal globular domain (NCI domain) and it is assembled into a network structure. This collagen is the major structural component in the basement membrane and is chemically and genetically distinct from collagen types I, II, and III, which are found mainly in interstitial connective tissue. However, little is known about type IV collagen of skin in patients with ALS.

Objectives: To study type IV collagen of serum and skin in ALS patients.

Methods: Serum levels of the 7S fragment of the N-terminal domain of type IV collagen (7S collagen) were measured in 18 ALS patients and 19 control subjects and type IV collagen immunoreactivity of skin in 8 patients with ALS (nos. 1 to 8) and 11 diseased control subjects (nos. 1 to 11) were studied.

Results: Serum 7S collagen levels in patients with ALS were significantly decreased ($P < 0.01$) as compared with those in diseased and healthy control subjects and were negatively and significantly associated with duration of illness ($r = -0.81$, $P < 0.001$). The basement membrane as well as blood vessels of skin in ALS patients was weakly positive for type IV collagen as compared with those of diseased control subjects. This weak immunostaining became more pronounced as ALS progressed. The optical density for type IV collagen immunoreactivity in ALS patients was significantly lower ($P < 0.001$) than in diseased control subjects and was significantly decreased with duration of illness ($r = -0.85$, $P < 0.01$). There was an appreciable positive correlation between concentrations of serum 7S collagen and the density for type IV collagen immunoreactivity in ALS patients ($r = 0.81$, $P < 0.02$).

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Discussion and Conclusions: The results concerning the decline of type IV collagen immunoreactivity of skin in patients with ALS might indicate a reduction of this protein content caused by degeneration process or a decrease of its synthesis, or both. There is an intimate relationship between changes of type IV collagen content of skin and serum 7S collagen levels in ALS and that the decreased amounts of serum 7S collagen may reflect, at least in part, a decreased type IV collagen immunoreactivity of skin in ALS. Thus, a metabolic alteration of type IV collagen may take place in the skin of ALS patients.

P99 MITOCHONDRIAL DAMAGE IN FIBROBLASTS OF PATIENTS WITH ALS

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Keywords: Mitochondria, Fibroblasts, DNA

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease hallmarked by a progressive loss of motor neurons. While at present the cause of neuronal cell death in ALS is unclear, the disease has been commonly regarded as a neurodegenerative disorder primarily involving the pyramidal motor system. Importantly, there is increasing evidence that disease-related degenerative changes also occur in extramotor areas of the CNS. Since sufficient fresh brain tissue of ALS patients is generally unavailable for comparison and multiple findings suggest that ALS is a multisystem neurodegenerative disease, skin fibroblasts were investigated.

Methods: To verify the putative impairment of mitochondrial function in fibroblasts of patients with ALS, the oxygen consumption (respiration rate) of fibroblast was measured at 37°C using an Oroboros high resolution oxygraph. Furthermore, mitochondrial DNA copy number analysis and deletions screening in fibroblast cultures were performed. Age matched healthy subjects served as controls.

Results: A statistically significant lower maximal rate was observed with NAD-dependent substrates glutamate and malate in the fibroblasts of ALS patients compared to controls (decrease >25%). The succinate supported maximal respiration rates in the fibroblasts from these patients were also significantly lower (decrease >20%). Deficiencies of respiratory chain complex I (NADH:CoQ1 oxidoreductase) and IV (cytochrome c oxidase, COX) were identified by determining the elevated flux control coefficients of respiration in titrations with specific inhibitors (amytal for complex I and azide for complex IV). Furthermore, in the fibroblasts of the investigated patients, diminished levels of mitochondrial DNA copies were observed.

Discussion and Conclusions: These results support the viewpoint that mitochondrial impairment is detectable in extracerebral tissue of patients with ALS. In addition to previous reports showing mitochondrial dysfunction in skeletal muscle of patients with ALS a similar defect can also be observed in fibroblasts. The mitochondrial alterations found may be explained by oxygen radical-induced damage of mtDNA.
These results show mild mitochondrial impairment in fibroblasts of patients with ALS. These findings may have implications for early diagnosis and for monitoring in the context of establishing a novel neuroprotective strategy for treating ALS.

P100 ANALYSIS OF OXIDATIVELY MODIFIED WT SOD1 IN PATIENTS' LYMPHOBLASTS: IMPLICATION FOR A TOXIC ROLE OF WT SOD1 IN SPORADIC ALS

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Keywords: sporadic, SOD1, lymphoblast

Background: The mechanisms involved in selective motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS) remain mainly unknown. 90% of ALS cases are sporadic (SALS). About 10% of cases are familial (FALS) and approximately 25% of FALS patients inherit autosomal dominant mutations in the gene encoding copper-zinc superoxide dismutase (SOD1). Recent studies suggest that Wild-Type SOD1 (WTSOD1) may represent a major target of oxidative damage in neurodegenerative diseases and that WTSOD1 may acquire aberrant and toxic properties similar to those of the mutated SOD1 (1) in at least a subset of SALS.

Objectives: To test the hypothesis that due to age and/or environmental associated oxidative damage, WTSOD1 undergoes post-translational and/or conformational modifications similar to those caused by disease-causative mutations and therefore WTSOD1 may be involved in SALS.

Methods: Lymphocytes from 3 FALS, 6 SALS and 5 healthy controls were immortalized using EBV virus and treated with 100 μM hydrogen peroxide (H_2O_2) for 4 hours. Immunoprecipitation: lymphoblasts were homogenized in Chaps buffer. After pre-clearing, samples were incubated with an anti-SOD1 antibody. The antibody-antigen complexes were precipitated and analyzed by Western blotting (WB). Confocal analysis: cells were plated on poly-L-lysine pre-coated slides and fixed using 10% paraformaldehyde. Samples were treated with a blocking solution (5% goat serum, in 0.1% Tween-PBS), incubated o/n with anti-SOD1 antibody and then with secondary antibody. Flow cytometry: Cells were collected, washed with PBS and fixed with 4% paraformaldehyde. Samples were treated with a blocking solution (0.5% BSA, in 0.1% Tween-PBS), incubated o/n with anti-SOD1 antibody and with secondary antibody.

Results: Using patients' lymphoblasts this study showed disease specific properties of WTSOD1. In a subset of SALS (characterized by an upper motor neuron disease onset) WB analysis of immunoprecipitates from cell lysates revealed that, after the exposure to H_2O_2, WTSOD1 forms an aberrant complex with Bcl-2, as already seen in spinal cord of patients carrying one SOD1 mutation (2). Confocal and flow cytometry analysis, at basal level and after H_2O_2 treatment, shows a higher fluorescence of WTSOD1 in FALS and SALS lymphoblasts. No differences in protein expression have been found by analyzing lymphoblasts total lysate by WB. This discrepancy may be due to a protein conformational change attributable to an altered pro-oxidant cell environment.

Discussion and Conclusion: These results suggest that at least in a subset of sporadic patients, WTSOD1 may acquire binding and toxic properties similar to those observed in mutant SOD1. Studies are underway to test whether these modifications are indeed toxic and if they participate in the pathogenic mechanism(s) underlying the disease in SALS.

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P101 THE EXPRESSION PATTERN OF TDP-43 IN CIRCULATING LYMPHOMONONUCLEAR CELLS IN ALS SUPPORTS A DOUBLE PATHOGENIC MECHANISM OF AGGREGATE DEPOSITION IN MOTOR NEURONS

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Keywords: TDP-43, Western blot, TARDBP gene mutations

Background: TDP-43 is a highly conserved and ubiquitously expressed protein predominantly localized in the nucleus. In ALS tissue, it is delocalized to the cytoplasm and undergoes accumulation and aggregation. Pathogenic missense mutations in TARDBP have been reported in ALS cases coexpressing the TDP-43 pathology of sporadic ALS.

Objectives: To test the hypothesis that the protein pattern profile shows similar features in ALS with and without mutations.

Methods: By Western immunoblotting, the pattern of TDP-43 in the cytosolic (CF) and nuclear (NF) fractions of circulating lymphomonocytes (CLM) in ALS patients with and without TARDBP missense mutations were analysed as well as in spinal cord and frontal neocortex of patients with sporadic ALS.

Results: In NFs from controls as well as ALS with or without TDP-43 mutation, TDP-43 protein was expressed in an approximately comparable amount as a protein with the predicted molecular mass of about 45 kDa. In CFs two protein bands sized around 43 kDa and 41 kDa were detected. The signals of the two bands were weak in controls and ALS patients without mutation, while either one or the other of the two bands prominently accumulated in the blots of ALS patients bearing different TARDBP gene mutations. The CFs blots from samples of spinal cord and frontal cortex of ALS patients without gene mutations were qualititatively different from blots of control tissue, being the TDP-43 45 kDa protein appreciable in ALS tissue only.

Discussion and Conclusions: The accumulation of one cytoplasmic form of TDP-43 in CLM appears to be linked to the gene mutation, presumably by impairment of proteolytic mechanisms involved in the nuclear import-export systems. The altered truncated forms of TDP-43 could promote aggregate formation (1); however, cytoplasmic aggregates are not found in CLM while they are prominent in motor neurons of ALS patients. Therefore, an additional dysfunction occurring in specific cells should contribute to the deposition of insoluble, ubiquitin-tagged protein aggregates in the cytoplasm of motor neurons. Reasonable candidates can be a locally restricted impairment of the ubiquitin-proteasome proteolytic system. The similar expression pattern of TDP-43 in CLM of both ALS patients with gene mutation and healthy relatives bearing the same mutation found is consistent with the double pathogenic mechanism.

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P102 SERUM URATE LEVELS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oxidative stress, urate, pathogenesis

Background: The pathogenesis of amyotrophic lateral sclerosis (ALS) remains unknown and multiple mechanisms (such as apoptotic pathway activation, excitotoxic aminoacids-mediated damage and oxidative stress) may be involved. Both *in vitro* and *in vivo* studies indicate that uric acid exerts antioxidant effects on neurons and several population-based studies have observed that low urate concentrations are associated with an increased risk for neurodegenerative disorders, as Parkinson's Disease and dementia.

Objectives: To determine if plasma urate concentrations are higher in ALS patients than healthy controls and to examine the relationship between urate concentrations and ALS.

Methods: A cross-sectional study was conducted to compare serum urate levels in 133 patients with ALS and 342 age/sex-matched controls. Patients were recruited among outpatients attending the ALS multidisciplinary clinic of Bari, while controls were patients referred to neurological clinics for their first visit contemporary to ALS patients. Subjects were excluded if they were affected by stroke, myocardial infarction, dementia, Parkinson's disease and other neurodegenerative disorders, that may be associated with changes in urate concentrations. Finally, subjects taking drugs that can increase uric acid or that can decrease urate measurements were not included in the study.

Results: Patients with ALS had lower median plasma urate levels (4.2 mg/dL (range: 1.4–8.5) versus 4.75 (range: 1.7–13.1; $P = 0.04$)), compared to controls, while age and sex distributions were similar. Univariate logistic regression revealed a direct association between plasma urate levels and presence of ALS (odds ratio; confidence interval: 0.3–0.98; $P = 0.04$). The relationship was confirmed even after correction for age and sex (odds ratio: 0.56; 95% confidence interval: 0.29–1.06; $P = 0.07$).

Conclusions: Plasma urate levels were significantly lower in patients with ALS compared to age and sex-matched controls. These findings suggest that urate may protect neurons against oxidative damage caused by reactive nitrogen and oxygen species involved in the pathogenesis of ALS.

P103 MACROPHAGE ACTIVITY MARKER SOLUBLE CD14 IS INCREASED IN PLASMA OF PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: monocyte/macrophage activation, lipopolysaccharide (LPS), soluble CD14 (sCD14)

Background: Previous studies have demonstrated elevated levels of abnormally activated monocyte/macrophage in patients with sporadic ALS (sALS). Significantly enhanced levels of plasma Lipopolysaccharide (LPS), a systemic macrophage activator were found in sALS patients. The increased LPS levels correlated with levels of abnormally activated monocyte/macrophages. Others have shown that injection of LPS into SOD1G37R ALS mice causes a dramatic shortening of their lifespan, suggesting that LPS mediated macrophage activation may exacerbate the pathogenesis of ALS. CD14, initially described as a specific receptor for LPS, is found in membrane-bound and soluble forms. CD14 mediates the interaction of LPS with CD14-bearing inflammatory cells (monocyte/macrophage) and is known to be an essential mediator of inflammation in innate host defense. Soluble CD14 (sCD14) also facilitates LPS activation of CD14-membrane negative cells such as endothelial cells, epithelial cells and astrocytes. As a marker for monocyte/macrophage activation and a mediator of LPS action, sCD14 can therefore be a possible biological marker in monitoring the inflammatory and immune activity in ALS.

Objectives: To quantify levels of plasma sCD14 in sALS patients as compared to control groups. To determine whether plasma sCD14 levels correlate with clinical stages of disease in sALS.

Methods: sCD14 ELISA was performed to quantify plasma sCD14 levels in heparinized blood samples from 15 sALS patients, 12 Alzheimer's (AD) patients, and 15 healthy controls (HC). Results from immune studies were evaluated in light of the severity of neurological impairment as determined by ALSFRS-R scores.

Results: Compared to HC (756 ± 183 ng/ml), significantly higher levels of plasma sCD14 were identified in sALS (1083 ± 243 ng/ml, $P < 0.01$) and AD (1194 ± 290 ng/ml, $P < 0.001$). Plasma sCD14 levels between two disease groups, sALS and AD, were similar. Similar patterns were observed in previous studies on plasma LPS. Significantly increased levels of plasma LPS were found in sALS and AD patients as compared to HC. Plasma sCD14 and LPS levels in sALS, AD and HC were significantly correlated ($r = 0.5497$, $P = 0.0002$). There was a trend of increased levels of plasma sCD14 with ALS disease severity: patients with more marked impairment had the highest scores (ALSFRS-R score = 25–36, $n = 8$) compared with those with mild impairment (ALSFRS-R score = 37–48, $n = 6$). Both groups were significantly higher than HC ($P < 0.01$ and $P < 0.05$ respectively).

Conclusions: This study reveals that plasma sCD14 levels are significantly elevated in patients with sALS and might be associated with ALS disease progression. Increased levels of sCD14 were significantly correlated with degree of plasma LPS. These findings not only demonstrate a role for peripheral immune activation in ALS pathogenesis but also suggest that plasma levels of sCD14 might be able to be used to monitor neurological dysfunction during progressive ALS disease. New approaches to down-modulate LPS-sCD14 induced monocyte/macrophage activation may provide strategies for therapeutic intervention in patients with ALS.

P104 ALTERED EXPRESSION OF THE C-TYPE LECTIN RECEPTOR MINCLE AND ITS TRANSCRIPTION FACTOR cEBP ß - ADDITIONAL EVIDENCE FOR THE INVOLVEMENT OF THE INNATE IMMUNE SYSTEM IN THE PATHOGENESIS OF ALS

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Keywords: mincle, cEBP ß, c-type lectin

Background: In general, innate immunity offers the main resistance against pathogens within the first minutes and hours of infection. It is executed by phagocytic cells and not stimulated by antigens or mediated by antibodies. In addition, there is evidence that mechanisms of innate immunity may play a role in neurodegenerative disease and propagate neuroinflammation.
The activation of phagocytes is mediated by a set of pattern-recognition receptors (PRRs). One of the main groups of PRR is the c-type lectin receptors. Recently, mincle (also called Clec4e or Clecsf9) has attracted attention within this group, since it has been shown to be a sensor for dead cells (1). Mincle is controlled by the transcription factor cEBP ß.

Objectives: To determine whether the expression of mincle and its transcription factor cEBP ß may be altered in the spinal cord of ALS-patients and SOD transgenic mice and may therefore be involved in the pathogenesis of ALS.

Methods: Microarray techniques as well as immunohistochemistry and immunoblotting were used to assess the quantity and cellular distribution of mincle, cEBP ß mRNA and protein levels.

Results: A prominent upregulation of mincle mRNA and protein was found in the spinal cord of ALS patients and SOD mice. Double-staining of mincle, together with glial markers revealed that most mincle expression is located in microglial cells. Furthermore, an altered translation and subcellular localisation of the mincle transcription factor cEBP ß was observed. Its mRNA levels were increased in SOD mice compared to control animals while cEBP ß protein levels in whole cell extracts were reduced in the ALS model. In contrast, nuclear extracts of SOD mice revealed higher cEBP ß protein levels compared to their non-transgenic littermates.

Discussion and Conclusions: Mincle has a dual role as a sensor of microorganisms as well as abnormal cells. Activation of this receptor results in the release of pro-inflammatory substances and induction of phagocytic activity. ALS damaged motoneurons may activate spinal microglia via mincle and induce inflammatory mechanisms which further propagate the disease. Dysregulation of its transcription factor cEBP ß has been described in Alzheimer's disease, and may thus contribute to pathological neurodegenerative processes.

Reference:

1. Yamasaki *et al.* Nat. Immunol 2008;9:1179–1188

P105 EXPRESSION AND CELLULAR LOCALIZATION OF TOLL-LIKE RECEPTORS (TLR) 2, 4 AND HIGH MOBILITY GROUP BOX 1 (HMGB1) IN AMYOTROPHIC LATERAL SCLEROSIS HUMAN SPINAL CORD

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Keywords: toll-like receptors, HMGB-1, neuroinflammation

Background: Increasing evidence indicates that inflammatory responses could play a critical role in the pathogenesis of motor neuron injury in amyotrophic lateral sclerosis (ALS). Recent findings have underlined the role of Toll-like receptors (TLRs) in the regulation of both innate and adaptive immunity in different pathologies associated with neuroinflammation. TLRs form a large family of evolutionarily conserved transmembrane glycoproteins considered to represent "sensors" of the innate immune system, mediating the sustained glia reactivity observed under pathological conditions. TLRs are also activated in response to tissue injury by endogenous molecules named "damage-associated molecular patterns" (DAMPs), such as the high mobility group box 1 (HMGB1). HMGB1 is a ubiquitous nuclear non-histone DNA-binding protein, critical for transcriptional regulation and can act as a pro-inflammatory mediator upon its extracellular release in response to cell damage as found in degenerating neurons.

Objectives: To investigate the expression pattern and cell specific distribution of TLR2, TLR4 and their endogenous ligand HMGB1 in spinal cord of patients with sporadic ALS.

Methods: Paraffin-embedded sections of spinal cord of both sporadic ALS patients (n = 12; of whom n = 6 rapid progressors and n = 6 long term) and controls (n = 5) were immunostained to assess the cellular localization of TLR2, TLR4 and HMGB1. The extent of immunostaining was assessed on the basis of morphology and distribution. Furthermore, real-time PCR was performed to assess TLR and HMGB1 expression.

Results: The immunohistochemical analysis of TLR2 and TLR4 showed increased expression in reactive glial cells in both gray (ventral horn) and white matter of ALS spinal cord. TLR2 was predominantly detected in cells of the microglia/macrophage lineage, whereas the TLR4 was strongly expressed in astrocytes. Real-time quantitative PCR analysis confirmed the increased expression of TLR2 at the mRNA level in patients with rapid disease progression. In control spinal cord, nuclear HMGB1 distribution has been observed in glial cells. HMGB1 immunostaining increased in the cytoplasm of reactive glia of ALS spinal cord, indicating a possible release of this molecule from glial cells.

Conclusions: These findings show increased expression of TLR2, TLR4 and HMGB1 in reactive glia in human ALS spinal cord, suggesting activation of the TLR signalling pathways. The activation of this pathway involving both microglia and astrocytes may contribute to the progression of inflammation, resulting in motor neuron injury. Future studies will increase our understanding of TLR-mediated neuroinflammation, allowing for possible therapeutic interventions to be proposed in patients with ALS.

P106 RETINOID SIGNALING ALTERATIONS IN PATIENTS WITH ALS

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Keywords: retinoid signaling, nuclear receptors

Background: The retinoid signaling pathway is critical during development with increasing evidence that it continues to play an important role in adulthood. With respect to ALS, studies have reported the differential expression of components of this pathway in ALS patients and in both rat and mouse models of ALS. In addition, rats that are fed a vitamin A deficient diet exhibited pathological features of ALS including motor neuron loss in the lumbar spinal cord.

Objectives: To characterize proteins of the retinoid signaling pathway in patients with ALS to determine if retinoid signaling is altered in the disease.

Methods: Human post-mortem lumbar spinal cord sections were obtained from sporadic ALS (SALS) patients (n = 20), familial ALS (FALS) patients (n = 4) and non-neurological disease controls (n = 9). Immunohistochemistry was used to determine the localization of cytoplasmic proteins and nuclear receptors. Spinal cord tissue homogenates from a subset of patients were used to investigate levels of cytoplasmic binding proteins in total spinal cord homogenates. Nuclear-enriched fractions were used for co-immunoprecipitation studies and electrophoretic mobility shift assays (EMSAs) to characterize protein-protein and protein-DNA interactions, respectively.

Results: Cellular retinoic acid binding protein-II (CRABP-II), the protein responsible for facilitating nuclear receptor interactions, exhibited a punctate and nuclear staining pattern in SALS motor neurons compared to a more diffuse cytoplasmic pattern in FALS and controls. Although the localization of retinoic acid receptor alpha (RARα) did not differ when SALS, FALS and controls were compared, SALS motor neurons had an increase in retinoic acid receptor beta (RARβ) nuclear localization. Activated caspase-3 immunostaining was negative in SALS motor neurons exhibiting increased RARβ nuclear localization. Interactions between CRABP-II and either RARα or RARβ did not differ significantly. Retinoic acid response element binding was also observed in nuclear-enriched fractions.

Discussion and Conclusions: These results indicate that retinoid signaling is altered in ALS. More specifically, differences with respect to RARβ suggest that retinoid signaling impacts predominantly SALS and that this is a pro-survival response. Increased nuclear immunoreactivity of RARβ in motor neurons of SALS patients suggests increased nuclear targeting/retention of RARβ. Lack of RARβ nuclear immunostaining in FALS suggests a difference in underlying disease mechanism although studies of additional FALS subjects are required to validate this observation. Nuclear RARβ could indicate transcriptional activation or repression; further studies will determine the functional impact of this transcription factor.

P107 DIFFERENTIAL SIRTUIN mRNA EXPRESSION PATTERNS IN ALS POST MORTEM TISSUE

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Keywords: sirtuins, transcription, neuropathology

Background: Transcriptional dysregulation has been shown both in human sporadic ALS and in the G93A mouse model. Alteration in transcriptional regulation is an important feature of neurodegeneration and neuronal apoptosis. Transcriptional activity can be modulated via deacetylation of histones by histone deacetylases (HDAC). HDAC enzymes can be grouped into four classes, class I (HDAC 1–3, 8), class II (HDAC 4–7, 9, 10), class III (syn. SIRT 1–7; sirtuins) and class IV (HDAC 11) with distinct localization, substrates and physiological roles. Sirtuins are involved in the regulation of longevity, protein aggregation, stress responses, mitochondrial function and inflammation. They therefore present an interesting therapeutic target in neurodegenerative diseases. In a cell-based model, SIRT 1 deacetylase and resveratrol, as a SIRT1-activating molecule, have already been shown to protect against neurodegeneration. In a previous study the mRNA expression levels of HDAC 1–11 was examined in ALS post mortem tissue. The comparison between ALS and control tissues revealed significantly reduced levels of HDAC 11 in ALS motor cortex and spinal cord, while HDAC2 mRNA expression was significantly increased in ALS spinal cord.

Objectives: To assess the distribution and expression levels of the HDAC isoforms class III (SIRT 1–7) in post mortem ALS and control brain and spinal cord.

Methods: *In situ* hybridization histochemistry (ISH) was performed on human post mortem motor cortex and spinal cord sections of ALS patients (n = 3) and age matched controls with no history of neurological disease (n = 3). mRNA expression was quantified macroscopically by densitometric analysis of digitized film autoradiograms, the expression at the cellular level was studied by liquid emulsion autoradiography.

Results: At the macroscopic level, an increased mRNA expression of SIRT 2 and 5 was found in post mortem ALS motor cortex as compared to control tissue while SIRT 6 levels appeared unaltered. Further analysis will provide data on potentially different expression patterns at the cellular level, focusing in particular on neuronal versus non-neuronal cells in the primary motor cortex and the ventral horn.

Discussion: Therapeutic interventions aimed at activating or blocking sirtuins could become helpful in the treatment of human diseases. This requires a more precise understanding of disease specific alterations. The precise characterization of ALS-related alterations in SIRT expression levels or cellular distribution patterns could allow for more disease-specific pharmacotherapeutic approaches.

P108 ROUGH ENDOPLASMIC RETICULUM ALTERATIONS IN THE MOTOR NEURONS OF THE SPINAL CORD IN SPORADIC ALS

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Keywords: rough endoplasmic reticulum, endoplasmic reticulum stress, ultrastructure

Background: Endoplasmic reticulum (ER) stress is triggered by the accumulation of unfolded or misfolded proteins in the ER lumen and causes a cellular stress response called the unfolded protein response (UPR). UPR protects the cell against the toxic increase of unfolded or misfolded proteins. ER stress has recently been reported to be implicated in ALS. However, it remains controversial as to whether ER stress can play a major role in the pathomechanism of neurodegeneration. So far, aggregated proteins associated with neurodegenerative diseases have not been found in the ER.

Objectives: To examine ultrastructural alterations of rough ER (rER) of the normal-appearing large motor neurons in search of early pathological changes and to clarify whether ER stress is involved in the pathomechanism of neurodegeneration of motor neurons in patients with sporadic ALS.

Methods: Immunohistochemistry was used to examine the anterior horns of lumbar spinal cord (L1-L5) in 12 ALS patients and 12 controls, using the polyclonal antibody against glucose-regulated protein 78 (GRP78). Electron-microscopy was used to study the large motor neurons of lumbar spinal cord (L1–L5) in 15 ALS patients and 15 controls.

Results: Motor neurons in controls as a whole did not show GRP78 immunoreactivity except for a few neurons in two controls (4 out of 4196 large motor neurons: 0.1%). In ALS patients, both normal-appearing and degenerated motor neurons were immunostained for GRP78 in 6 patients, which is more frequent than in the controls (Chi-square test, $P = 0.04$). In ALS patients, 39 out of 1404 large motor neurons were positively immunostained (2.8%) and GRP78 immunopositive motor neurons tended to be more frequently observed than in the controls (GLM Procedure, SAS system, $P = 0.08$). Electron-microscopically, various kinds of rER alterations were observed: accumulations of granular, amorphous or vesicular materials in the dilated ER lumen (3 out of 15 patients), extended waving membranous structures resulting from ER membranes without membrane-bound ribosomes (one patient), lamellar bodies (3 patients), electron-dense material resembling Bunina bodies (5 patients), Hirano bodies directly connected to ER (1 patient), honeycomb-like structures (2 patients), membrane-particle complex (1 patient) and colloid inclusions (3 patients). In the controls, lamellar bodies (1 case) and colloid inclusions (another case) were observed.

Conclusions: Increased GRP78 immunoreactivity in motor neurons and the accumulation of granular, amorphous or vesicular materials in the dilated ER lumen suggest that the UPR is activated and ER stress is involved in the pathomechanism of neurodegeneration of motor neurons in patients with sporadic ALS.

P109 QUANTITATIVE ANALYSIS OF FMO GENE mRNA LEVELS IN ALS HUMAN BRAIN

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Keywords: FMO (Flavin-containing monoxygenases), qPCR, gene expression

Background: Flavin-containing monooxygenases (FMOs) are a family of microsomal enzymes that catalyze the oxygenative metabolism of a variety of nucleophilic heteroatom-containing xenobiotics in detoxication processes. Recent results have shown a relationship between Amyotrophic Lateral Sclerosis (ALS) and FMO genes. ALS is an adult-onset, progressive and fatal neurodegenerative disease and FMO changes may constitute new biomarkers for this condition.

Objectives: To examine FMO expression in human brain subregions defined as cervical cord, medulla, cerebral cortex and cerebellar motor cortex.

Methods: A Real-Time Sybr Green qPCR was used for expression analysis. Normalization was optimized using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene.

Results: FMO genes were detectably expressed in both control and ALS tissues. The differences in expression was generally higher in the ALS samples than in control tissues, with the largest increases in FMO expression detected in cervical cord. FMO5 in ALS was one hundred-fold greater than in control samples ($P < 0.001$). In medulla both FMO 1 and 5 were expressed at significantly greater amounts in ALS than in controls ($P < 0.001$ and $P < 0.05$). FMO1 and FMO3 mRNA levels were significantly greater in ALS than in control cerebral cortex samples ($P < 0.01$ and $P < 0.001$, respectively). Difference in expression between ALS and controls in motor cerebellar cortex was not significant.

Discussion and Conclusions: Altered FMO gene expression could be due to the involvement of FMO in oxidative-redox systems, as described for the yeast flavin-containing monooxygenase, yFMO that is vital to the "reductive stress" response. Up-regulation of FMO genes in ALS could be a reply to oxidative stress. It is not known if one or more FMOs play a role in the cellular response to oxidative stress but FMO are certainly involved in the general oxygenative/reductive tone of the cell. Although the precise pathogenic link is presently unknown, these findings of altered FMO expression suggest a role of FMO genes in ALS.

P110 LIGAND MODULATION OF CATALYTIC PROPERTIES OF IRON REDOX ACTIVITY IN CEREBROSPINAL FLUID OF ALS PATIENTS

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Keywords: cerebrospinal fluid, iron redox activity

Background: Extensive data show that redox-active metal iron plays a considerable role in pathogenesis of amyotrophic lateral sclerosis (ALS). Neurodegeneration is also related to a promoted production of H_2O_2, which could set up the conditions for the generation of highly damaging hydroxyl radicals ($^{\bullet}OH$) via the Fenton mechanism. Although it is known that total concentration of iron in CSF of ALS is close to normal values, there are some ambiguities about the role of ligand modulation of iron activity in CSF.

Objectives: To explore ligand modulation of catalytic properties of iron redox activity in the CSF of ALS patients using ethylenediaminetetraacetic acid (EDTA) and diethyldithiocarbamate (DDC).

Methods: The current study was performed using 15 recently diagnosed sporadic ALS (SALS) patients with a clinical status consistent with probable or definite ALS according to El Escorial criteria. None of the SALS patients had blood-CSF barrier dysfunction.
Electron Paramagnetic Resonance (EPR) spectroscopy and spin-trap BMPO (5-tert-Butoxycarbonyl-5-methyl-1-pyroline-N-oxide) were used to determine production of $^{\bullet}OH$ radicals in CSF treated with H_2O_2 (1 mM) and Fe^{3+} (final concentrations: 10 and 100 μM). Iron was applied free or in complex with EDTA. The effects of the chelating agent, DDC (500 μM) on the catalytic properties of iron in CSF were also evaluated. The intensity of EPR signal of BMPO/OH adduct was used as a measure of level of $^{\bullet}OH$ production in each investigated system.

Results: Different intensities of BMPO/OH signal were registered using EPR spectroscopy: 1) system with only H_2O_2 (20.0 ± 2.8); 2) system with Fe^{3+} (10 μM) and H_2O_2 (21.2 ± 10.7); 3) system with Fe^{3+} (100 μM) and H_2O_2 (23.0 ± 3.5); 4) system with Fe^{3+}EDTA (100 μM) and H_2O_2 (156.0 ± 7.2); and 5) system with Fe^{3+}EDTA (100 μM) and DDC (500 μM) and H_2O_2) (21.1 ± 3.4). It is obvious that the application of free iron even at high concentrations did not lead to increased $^{\bullet}OH$ generation in CSF of ALS. On the other hand, we noticed that iron in complex with EDTA, provoked significant increase of generation of $^{\bullet}OH$ which was not registered when DDC was added to this complex. Therefore, DDC diminished catalytic activity of iron in complex with EDTA in CSF.

Discussion and Conclusions: This model shows that normal total concentration of iron in CSF of ALS patients does not exclude the presence of ligands which could seriously modify iron catalytic activity and lead to oxidative damage of nervous tissue *in vivo*. DDC as chelating agents are effective against prooxidative activity of iron and may be adequate for preventing dangerous radical production in the pathophysiological settings of ALS.

P111 ACCUMULATION OF TDP-43 IS COMMON IN MYOPATHIES WITH RIMMED VACUOLES, BUT ABSENT IN MUSCLE IN PATIENTS WITH ALS

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Keywords: TDP-43, muscle, rimmed-vacuoles

Background: Polymyositis and dermatomyositis are considered to be pure inflammatory myopathies. In contrast, sporadic inclusion body myopathy (sIBM) is considered to also have primary degenerative characteristics. Muscle biopsies of patients with ALS lack either inflammatory or primary myodegenerative changes. The pathology of many neurodegenerative diseases is frequently characterized by the formation of protein

aggregates, such as beta-amyloid, tau, alpha-synuclein etc. These protein aggregates have been described in sIBM. The recently described TAR DNA-binding protein 43 (TDP-43) is a nuclear protein that is found in neurons in pathological aggregates in the cytoplasm and to a lesser degree intranuclear in MND, frontotemporal dementia (FTD) or the combination of FTD-MND. TDP-43 positive inclusions were demonstrated in FTD with inclusion body myopathy caused by mutation in the VCP gene, as well as in sIBM.

Objectives: To investigate whether aggregation of TDP-43 is present in muscles of patients with ALS, myopathies with rimmed vacuoles and inflammatory myopathies.

Methods: Frozen sections of muscle biopsies from patients with ALS (n = 3), neurogenic atrophy (n = 1), metabolic mitochondrial myopathy (n = 5), FSHD (n = 2), Duchenne's muscular dystrophy (n = 3), Becker's muscular dystrophy (n = 1), congenital central core disease (n = 5), sIBM (n = 18), dermatomyositis (n = 3), and polymyositis (n = 5) were compared to those with morphological normal biopsies (n = 5). Hematoxylin-phloxin staining was used for morphological analysis. Immunohistochemistry was performed with antibodies against TDP-43 and ubiquitin.

Results: Cytoplasmic TDP-43 positive aggregates were found in rimmed vacuoles in most cases with sIBM (14/18, 77.8%). Positivity was observed in various degrees in rimmed vacuoles, but also sometimes subsarcolemmal in small fibers. Coincidental ubiquitin positivity was frequently observed. Muscle biopsies of patients with ALS, dermatomyositis, polymyositis, all non-rimmed vacuolar myopathies and normal biopsies did not exhibit pathological TDP-43 staining.

Discussion: In this study the presence of TDP-43 positive aggregates in sIBM was confirmed, reflecting the degenerative character of sIBM. Other inflammatory myopathies seem to be negative. Moreover, no TDP-43 positive accumulations were found in muscles from patients with ALS. TDP-43 may be a useful marker to detect small rimmed vacuoles in sIBM.

Conclusion: Accumulation of TDP-43 is common in myopathies with rimmed vacuoles, whereas it is absent in muscle biopsies of patients with ALS.

P112 FUS PROTEIN AGGREGATES IN SPORADIC INCLUSION BODY MYOSITIS

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Keywords: FUS, TDP-43, VCP

Background: Sporadic inclusion body myositis (sIBM) is the most common muscle disease causing progressive muscle weakness and ultimate paralysis in people 50 years of age or older. Its pathogenesis is unclear and there is no primary treatment. Pathological hallmarks include cytoplasmic rimmed vacuoles, T-cell infiltration and deposits of a variety of proteins like amyloid-beta, tau, valosin-containing protein(VCP) and tar DNA-binding protein (TDP-43) and are pathogenic in neurodegenerative diseases. It is an ongoing debate as to whether sIBM is of an autoimmune origin or a degenerative disease. VCP mutations cause inclusion body myopathy with early-onset paget disease and frontotemporal dementia. TDP-43 is mutated in amyotrophic lateral sclerosis (ALS) and presents as a major component of protein aggregates in several neurodegenerative diseases. Very recently FUS (also known as translocation in liposarcoma or TLS) mutations were reported in familial ALS (FALS). FUS and TDP-43 are both RNA-binding proteins and might be involved in closely related physiological and pathological processes.

Objectives: To explore the involvement of FUS in sIBM and its interaction with other neurodenerative proteins.

Methods: sIBM cases included in this study were thoroughly evaluated and documented for degree and progression of muscle weakness. All cases were pathologically confirmed. Frozen muscle biopsy tissues were used for immunohistochemistry (IHC) and confocal microscopic examination. Normal human muscle biopsies were used as controls. IHC used the biotin-avidin system and confocal analysis was conducted with an indirect immunoflourescence assay.

Results: 10 sIBM muscle biopsies were examined with 3 normal muscle biopsies as controls. sIBM muscle showed atrophy, T-cell infiltration and rimmed vacuoles. Vacuoles were stained with SMI31 and 4G8 which recognize phosphorylated PHF and beta-amyloid respectively. Rimed vacuoles showed strong immunoreactivity with TDP-43, VCP and FUS. FUS and TDP-43 also stained the nucleus, but VCP was more confined to vacuoles in sIBM muscle biopsies. Confocal analysis showed co-localization of FUS with TDP-43 and VCP in the vacuoles.

Discussion and Conclusions: This is the first documentation of aggregation of FUS proteins in sIBM muscle fibers and its interaction with TDP-43 and VCP in sIBM. FUS is mutated in ALS and fused with oncogenes in Ewing's sarcoma and acute myeloid leukemia. The presence of FUS and its interaction with TDP-43 and VCP in sIBM muscle suggest that degeneration might be a more significant factor in the pathogenesis of sIBM which has implications in developing new therapy options. The involvement of FUS in myopathy also suggests its broader pathological role beyond neurodegeneration.

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THEME 5 GENETICS

P113 THE ALS ONLINE GENETICS DATABASE, ALSOD

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Keywords: genome wide study, database, bioinformatics

Background: The ALS Online Genetics Database, ALSOD (http://alsod.iop.kcl.ac.uk/), is a central repository for genetic information on ALS. The initial aim of this database was to discover genotype: phenotype (G2P) correlations for SOD1 mutations. There has been considerable progress both technologically and scientifically since ALSOD was originally setup in 1999, presenting an opportunity for the collection and presentation of ALS genetic data in a single repository. The database is being transformed to meet these challenges.

Objectives: To provide a single, continuously updated central repository summarizing the current state of ALS genetics using open source standards and providing a service to the ALS research community, including automated meta-analysis and integration of data from linkage and association studies.

Methods: The requirements of the ALS genetics research community were collected from the ALSOD feedback page. The database schema was restructured to allow for flexibility and expansion by changing table designs, rewriting queries and implementing appropriate stored procedures. Codes and scripts were written in programming languages like javascript, XML, C#, T-SQL and VB.NET integrated under the ASP.NET platform.

Results: The user registration process and data submission methods have been simplified and streamlined. ALSOD has been accessed over 25,000 times so far this year by users from 80 countries, with 8,226 accesses in the month of March alone. An overview of key published studies for approximately 40 ALS-related genes http://alsod.iop.kcl.ac.uk/overview/index.aspx is now included. Bioinformatics and analysis tools such as PLINK and Haploview have been integrated into the web-pages. Existing genome-wide association study data have been collected for meta-analysis and on-the-fly meta-analysis in which unpublished user-data is combined with existing studies confidentially and the result fed back in minutes. Links to gene variants, gene databases and relevant publications have been manually added to web-pages, but this process will be automated. Google AJAX search and Google Earth API have been combined to overlay geographical SOD1 mutation on the globe. This will be extended to other genes.

Discussion and Conclusions: Advances in genetics and the fast pace of publications mean that a central resource summarizing all available data in an easily digestible form is essential. Integration with existing databases and analysis software means that ALSOD is a powerful resource for exploring existing genetic information for ALS. New tools are in development and these will be made live after initial beta-testing is passed. We welcome ideas for development or the addition of open source code from the user community. ALSOD is widely used by the ALS genetics research community. We aim to make it an indispensable tool for ALS research.

P114 AN INTERNET-BASED APPROACH TO GENETIC DATA DISCOVERY IN ALS

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Keywords: internet, genetic database, SOD1

Background: About 10% of ALS cases are inherited or familial (FALS). Mutations in genes such as SOD1, VAPB and FUS have been identified. Although registries such as ALSOD exist for researchers and genetic counselors, they are only local efforts to collect phenotypic data and patients themselves have no systematic data sources with which to predict their outcomes.

Objectives: PatientsLikeMe.com, a novel disease-focused social network, was utilized to investigate the variability in age of onset, clinical manifestations and disease progression due to different ALS-causing mutations and other demographic factors. The objectives are to a) give FALS patients the opportunity to share information with others with the same genetic mutation, b) to aggregate phenotypic data about specific mutations to further research.

Methods: Genetic capture capabilities were added to PatientsLikeMe.com and all active members of the ALS community were queried as to their genetic mutation, if known. A genetics search engine was launched on the site so patients could identify others with the same mutation and share information. Demographic and clinical features of the mutation carrying cases were summarized for each mutation and were compared with the published literature.

Results: Fifty percent of self-identified FALS patients responded to the request to tell PatientsLikeMe any genetic information they had on their disease. Mutations were also reported by sporadic ALS patients without a family history, including negative SOD1 results. Seventeen ALS community members with 12 different mutations entered in their ALS genetics. The most frequently reported mutations were SOD1 A4V, SOD1 D90A and the VAPB mutation P56S. Preliminary analysis indicates that A4V and D90A progression curves match the published literature, while new information has been learned about the progression curves of lesser-known mutations such as L144P, L144F and E49K.

Conclusions: This is the first worldwide online study on the variation in ALS gene mutations. The phenotypic variability between different SOD1 and VAPB mutation-carrying patients in this study is dramatic. Atypical clinical features delayed diagnosis in some cases. The phenotypic variability underlines the hypothesis that ALS is a biologically heterogeneous disorder in which genetics, environment and potentially several other factors shape clinical phenotype.

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P115 TOWARDS DEFINING A MICRORNA (miRNA) PROFILE FOR ALS

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Background: It has been previously found that the steady state levels of the low molecular weight neurofilament (NFL) mRNA are suppressed in sporadic ALS (sALS) that this suppression is related to an alteration in NFL mRNA stability and that mtSOD1, 14-3-3 and TDP-43 proteins are all regulators of NFL mRNA stability. It has also been observed that TDP-43 is predominantly associated with NFL mRNA in P-bodies, suggesting a preferential shift to a degradative pathway. Therefore, the microRNA (miRNA) expression prolife in ALS spinal cords was examined to determine whether it would favor the movement of NFL mRNA into either a translationally silent RNA granule (stress or transport granules) or into a degradative granule (P-bodies). MiRNAs are an emerging class of small non-coding RNAs capable of negatively regulating gene expression. MiRNAs can either translationally silence mRNA through an imperfect compliment to the miRNA recognition element (MRE) or lead to degradation through complete complimentarity.

Objectives: To characterize the expression profile of miRNA that interact with NFL mRNA 3'UTR and determine whether this profile differs between ALS and control.

Methods: Two different prediction algorithms (Target Scan, MiRanda) were used to develop a panel of highly conserved miRNAs that recognize the human NFL mRNA 3'UTR. This yielded 19 distinct miRNAs. miRNA was extracted from ventral lumbar spinal cord lysates of control, sALS, or familial ALS (fALS (with or without mtSOD1 A4T expression)) using the MirVana miRNA isolation kit and then qRT PCR was used to assay for the individual miRNAs. Copy levels were quantified against a known standard and the expression level were described as high (10^7–10^9 copies), intermediate (10^4–10^6 copies), low (10^1–10^3 copies) or absent. Differences between miRNA expression levels amongst cases were analyzed using RealTime StatMiner version 3.0 (Integromics).

Results: Four distinct expression levels were observed amongst the candidate miRNAs, including the following: high (miR23, miR25, miR30d, miR30e, miR92, miR195, miR214), intermediate (miR30b, miR183, miR188, miR367, miR452), low (miR32, miR192, miR215, miR323, miR363, miR380-5p) or absent (miR224). The expression levels of several individual miRNAs also differed significantly amongst the ALS cases. No difference was observed in expression levels between ALS and control for miR192, miR23, miR25, miR30d, miR30e and miR224. For fALS not linked to SOD1, a significant increase in miR32 and miR380 was observed as compared to control, sALS and mtSOD1 fALS, while miR195 was selectively decreased. Conversely, for mtSOD1 fALS, miR215 was selectively increased and miR188 decreased relative to all other cases. miR183 was selectively reduced in both sALS and mtSOD1 fALS relative to both control and non-mtSOD1 fALS. For sALS, miR323 was selectively reduced.

Conclusions: These observations lend support to the hypothesis that a miRNA expression profile for ALS spinal cord can be identified.

P116 MAPPING RUNS OF HOMOZYGOSITY IN IRISH SPORADIC ALS PATIENTS

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Keywords: homozygosity mapping,recessive mutations,population genetics

Background: Previous studies using genome-wide single nucleotide polymorphism (SNP) data have demonstrated that runs of homozygosity (ROHs) are common in humans, especially in homogenous populations such as the Irish. This is due to a common ancestry for the genomic region containing the ROH. Mapping ROHs in Irish ALS patients and in controls may indicate regions containing previously-unidentified recessive mutations that may be of pathological significance to the disease.

Objectives: To identify shared ancestral segments unique to Irish sporadic ALS (SALS) patients.

Methods: ROHs were determined using the computer programme PLINK in a total of 323 Irish SALS patients and 338 controls, genotyped using the Illumina 550k and 610k SNP arrays. These were considered recurrent when observed in more than two patients and ALS-specific when they did not overlap with ROHs observed in controls.

Results: There was no significant difference in the number and average length of ROHs between cases and controls. However, the number of recurrent ALS-specific ROHs was greater than the number of recurrent control-specific ROHs ($P < 0.001$). ROHs were mapped showing unique evidence of shared ancestry in at least five ALS patients. Several of these genomic regions contained biologically plausible candidate loci for ALS pathogenesis including a glutamate receptor, two potassium channels and several genes involved in neuronal integrity and signal transduction.

Discussion and Conclusions: By mapping ROHs in Irish ALS patients, several biologically plausible candidate genes have been identified. Deep re-sequencing of these regions may reveal recessive mutations conferring ALS susceptibility.

P117 ESTABLISHING BIOMARKERS FOR DISTINCT GENETIC AND DISEASE SUBTYPES USING GENE EXPRESSION PROFILING OF FIBROBLASTS FROM MOTOR NEURON DISEASE PATIENTS

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Keywords: fibrobalsts, microarray, TARDBP

Background: The term motor neurone disease (MND) is used for a collection of neurodegenerative disorders affecting the motor system. Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease affecting both the upper and lower motor neurones (UMN/LMN), usually associated with a disease duration of 2–3 years. Primary lateral sclerosis (PLS), affecting only UMN, has a slower progression of 10–15 years. It is unclear whether these are distinct disorders or part of a spectrum of neurodegenerative diseases. ALS is

usually sporadic, although 5–10% of cases are familial, with mutations commonly found in SOD1, TARDBP and FUS/TLS genes. PLS is predominantly a sporadic disease, although mutations in alsin are associated with juvenile PLS. Peripheral samples (blood and fibroblasts) have been used to investigate both pathophysiological mechanisms and biomarkers of neurological disease. Fibroblast cultures derived from patients offer advantages over blood samples as they provide a source of material for downstream functional studies following microarray analysis. In MND, fibroblasts from patients are more sensitive to oxidative stress than controls.
This study tests the hypothesis that distinct gene expression profiles are found in fibroblasts from PLS, sporadic ALS (SALS) and specific genetic subgroups of ALS.

Objectives: 1) To generate gene expression profiles from PLS, SALS, TARDBP-ALS and controls, 2) To determine differentially expressed genes which are specific for each of the disease subtypes, 3) To validate changes in informative genes by QPCR, 4) To establish the effectiveness of using these genes as diagnostic indicators in a second cohort of patients.

Methods: Fibroblast cultures were established from skin biopsies from 12 PLS, 12 SALS, 3 TARDBP-ALS cases and 12 neurologically unaffected controls. Total RNA was extracted, biotin-labelled and hybridised to the Human Genome U133 Plus 2.0 GeneChips to obtain expression levels of 38,500 genes. GeneChips were normalised using PLIER and genes differentially expressed >2-fold, $P<0.05$ identified using ArrayAssist. Functional annotations used DAVID and NetAffx.

Results: Expression profiles have been generated from 6 PLS, 6 SALS, 3 TARDBP-ALS and 6 controls. Pair-wise analysis has shown 583 transcripts are differentially expressed between PLS v controls, whereas 990 transcripts are differentially expressed between SALS and controls and 1, 146 transcripts between TARDBP-ALS and controls. Of these, 367 are differentially expressed in both ALS groups and 187 are differentially expressed in both the PLS and SALS groups. Biological processes affected in ALS include RNA processing and the cytoskeleton, whilst in PLS, ion channels and cell development are affected. QPCR of key genes in these categories will be presented.

Discussion and Conclusions: Identification of the most informative discriminatory genes for these subtypes, whatever their biological function, can serve as potential biomarkers for disease, as well as providing clues for the pathological mechanisms of neurodegeneration.

P118 GENE EXPRESSION PROFILING IN SKELETAL MUSCLE IN AMYOTROPHIC LATERAL SCLEROSIS: A TOOL FOR BIOMARKER DISCOVERY

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Keywords: muscle, transcriptome, biomarker

Background: In ALS, molecular alterations in skeletal muscle are a consequence of denervation and may also contribute to the non cellular autonomous degeneration of motoneurons. Studying the gene expression profiling in skeletal muscle biopsies represents a valuable approach to the discovery of potential diagnostic markers or markers of disease progression.

Objectives: To compare the muscle transcriptome of patients with sporadic ALS with that of healthy subjects as a means to identify new potential markers of ALS.

Methods: To provide an accurate study of gene expression on a large scale, Affimetrix GeneChips® high-density oligonucleotide microarrays were used and GeneSpring® statistical analysis was performed for data mining.

Results: According to the abduction score in the arm prior to biopsy (which correlated well with the degree of muscle atrophy), the ALS patients were classified in two groups: patients with normal muscle strength and patients with diminished muscle strength. Then, transcripts were selected whose averaged expression signals changed at least 2-fold between ALS and control subjects and between the two groups of ALS patients. Firstly, 25 transcripts were identified that were significantly regulated only in ALS patients with normal muscle strength, thus representing unique markers of a very low level degree of atrophy. Secondly, 70 transcripts were isolated that were regulated only in ALS with diminished muscle strength and hence interpreted as markers of an advanced stage of disability and atrophy. Finally, 39 transcripts were identified whose expression, as regarded patient by patient, correlated very significantly with the arm abduction score and thus was considered as a measure of the progression of the pathological process.

Conclusions: This study reveals distinctive subsets of genes that characterize patients with different degrees of muscle strength disability and muscle fiber atrophy in the biopsied sample. These sets of genes are promising candidates to be further explored and validated as molecular biomarkers for ALS.

P119 MICROARRAY ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: diagnosis, lymphocytes, microarrays

Background: Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is characterized by the progressive loss of motor neurons, leading to paralysis and death from respiratory failure. ALS is an incurable adult-onset disease with unknown cause. Currently, early diagnosis provides our best hope to delay disease progression by early treatment and with supportive care to improve quality of life. Specific genes expression patterns in leukocytes have previously been found for CNS disorders. So far, no ALS-specific expression patterns exploitable towards early diagnosis have been found in peripheral blood leukocytes from ALS patients.

Objectives: To perform gene expression profiling by microarray analysis on purified peripheral blood lymphocytes (PBLs) from patients diagnosed with definite ALS versus healthy controls (HC).

Methods: Blood samples from ALS patients (n = 11) diagnosed with definite sporadic ALS and HC (n = 11) were collected at Carolinas Neuromuscular ALS/MDA Center. PBLs were isolated using Ficoll-Paque gradient centrifugation followed by Percoll step-gradient centrifugation. The common reference design was used for sample assignment in the dual color mode of expression assay on the 4X44K Whole Human Genome Microarrays (Agilent) to analyze ~40,000 transcripts. Microarray experiments were performed in which each of the 22 RNA samples (HC and sALS) was co-hybridized with RNA from the HC reference pool. Background subtracted raw data were normalized using MIDAS pipeline (TM4, TIGR Genomics): total intensity normalization-LocFit(LOWESS)-standard deviation regularization-low intensity trim. Permutation T-Test with adjusted Bonferroni correction and SAM were performed on Log_2 ratios in TMeV/TM4.

Results: A total of 167 genes were found to be differentially expressed by permutation-based T-test (fold change (FC) range 1.2–3.2) including TARDBP/TDP43 (1.3 FC). SAM declared 215 significant genes with local FDR (q values) less than 10% (FC range 1.7–4.4). The intersection between SAM and permutation T-Test, which did not include TARDBP/TDP43, consisted of 37 differentially expressed genes (10 upregulated). Among upregulated genes, known genes included RAD54B, a DNA repair and recombination protein (3.1 FC, q < 1%), KLRA1, a natural killer cell receptor (2.6 FC, q < 1%) and the amyloid beta (A4) precursor protein APP involved in Alzheimer disease (1.9 FC, q = 7.8).

Discussion and Conclusions: TARDBP, that has been implicated in ALS pathogenesis, was found to be marginally differentially expressed, possibly due to signal dilution of a specific lymphocyte subpopulation. These data also suggest that upregulation of APP is likely to result from neurodegeneration reflected in peripheral lymphocytes. The upregulation of KLRA1 is intriguing as it suggests activation of natural killer cells normally representing 15% of PBLs or activation of a subpopulation of T-cells. Validation of these results on a larger sample size (including early ALS patients) by real-time RT-PCR, immunocytochemistry and FACS analysis is underway to determine if there are lymphocyte subpopulations specific to ALS patients.

P120 GENE EXPRESSION PROFILING OF BLOOD FROM PRIMARY LATERAL SCLEROSIS PATIENTS TO DETECT BIOMARKERS AND PATHWAYS INVOLVED IN THE PATHOGENIC DISEASE PROCESS

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Keywords: PLS, microarray, biomarkers

Background: Primary Lateral Sclerosis (PLS) is a rare, sporadic, adult-onset variant of motor neuron disease (MND) in which upper motor neurons (UMN) degenerate. PLS often has a slower and more benign course than the amyotrophic lateral sclerosis (ALS) variant of MND. Clinically it is difficult to determine whether a patient over time will go on to develop a more aggressive ALS phenotype. In addition there is a debate as to whether PLS is a distinct disease entity or at one end of a spectrum of MND. Peripheral blood is a desirable source for gene expression profiling as microarray studies show that on a transcriptional level, whole blood shares significant similarities with multiple CNS tissues. Thus identifying gene expression profiles in the blood of PLS and ALS patients may reveal biomarkers and pathways specific to the PLS disease state, thereby allowing differentiation from ALS cases.

Objectives: To compare expression profiles of peripheral blood from PLS cases, neurologically normal controls and disease (ALS) controls to elucidate biomarkers of PLS.

Methods: RNA was extracted from peripheral blood of 14 PLS patients, 13 ALS patients and 14 age and sex matched healthy volunteers. Following removal of globin RNA, the samples were amplified and hybridized onto U133A Plus 2.0 Human GeneChips, allowing the simultaneous detection of 47,400 transcripts corresponding to 38,500 genes. Transcripts were identified as being differentially expressed between patients and controls if they showed >2-fold and $P < 0.05$ using the PLIER (Probe Logarithmic Intensity Error) algorithm. Genes were consequently classified into functional pathways based on their molecular function using their GO terms.

Results: Comparing PLS cases and controls, 2,488 genes were found to be differentially expressed with 1,652 up regulated and 836 down regulated in patients versus controls. Key functional pathways altered in the PLS disease state include energy metabolism alterations in glycolysis, glucose uptake and insulin signalling. In addition, the mTOR pathway controlling protein synthesis and MAPK acting as a feedback mechanism to the mTOR pathway are altered. Comparing ALS cases and controls, 2,113 genes were differentially expressed with 1,772 upregulated and 341 down regulated in patients versus controls. Further analysis is being carried out to identify pathways involved in ALS and to compare them with PLS specific pathways.

Discussion and Conclusions: This study offers a novel approach to the identification of biomarkers in MND and suggests that it may be possible to differentiate its subtypes. Findings of altered energy metabolism in PLS patients may provide insights into the energy homeostasis abnormalities observed in MND patients in previous studies.

P121 SPG4 MUTATIONS IN A COLLECTION OF JAPANESE PATIENTS WITH HEREDITARY SPASTIC PARAPLEGIA

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Keywords: AD-HSP, SPG4

Background: Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a group of genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity and weakness of the lower limbs because of axonal degeneration of the corticospinal tracts. The most common form of AD-HSP, accounting for approximately 40% of affected individuals, is due to mutation in the *SPG4* gene. *SPG4* encodes spastin, a 616 amino acid protein belonging to the AAA family (ATPases Associated with diverse cellular Activities).

Objectives: To investigate the *SPG4* mutation profile in Japanese patients with AD-HSP and to describe them in the context of the corresponding clinical phenotype.

Methods: Informed consent, blood samples and clinical evaluations were obtained from forty AD-HSP patients. Genomic DNA and total RNA were extracted from lymphocytes using standard procedures. Genomic DNA was analyzed by direct sequencing of all exons of *SPG4* and mRNA was analyzed by reverse transcription polymerase chain reaction (RT-PCR) analysis on the patients with *SPG4* mutations.

Results: Seventeen patients had *SPG4* mutations; five missense mutations (29%), four nonsense mutations (24%), four splicing mutations (24%), three frameshift mutations (17%) and one in-frame deletion mutation (6%). During the RT-PCR analysis of mRNA, exon 8 was identified as skipping on two patients, which was one of the splice variants of spastin.

Discussion and Conclusions: The *SPG4* mutations accounted for approximately 42.5% (17/40) of the mutations in AD-HSP, which was the same as the estimated 40% linked to this locus. Exon deletions of SPG4 were a frequent cause of HSP in previous studies using the MLPA (multiplex ligation-dependent probe amplification) method, but exon deletions could not be detected apart from splice variants.

P122 IDENTIFICATION OF A NOVEL MISSENSE MUTATION IN THE SPG4 GENE IN A PATIENT WITH A SUSPECTED MOTOR NEURONE DISEASE

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Keywords: spastin, SPG4, HSP

Background: Hereditary spastic parapareses (HSP) represent a group of rare neurodegenerative disorders clinically and genetically heterogeneous, characterized by progressive spasticity and weakness of the lower limbs. Dominant, recessive and X-linked forms have been described. Autosomal dominant inheritance accounts for approximately 70–80% of all cases, whereas autosomal recessive HSP is considered a rare condition. Mutations in the spastin gene (SPG4) are the commonest cause of HSP accounting for up to 40% of autosomal dominant cases. More than 150 spastin mutations have been described to date.

Objectives: To perform a mutational analysis in the SPG4 gene in a patient with a suspected motor neurone disease.

Methods: The patient was a 49-year old female who developeda rapidly progressive spastic paraparesis at 46 years of age. Limb EMG, transcranial magnetic stimulation and somatosensory evoked potentials, brain and cervical spine MRI, blood assays of lactic acid, antiganglioside, antisulfatide and anti-MAG antibodies and circulating oxidative stress markers and CSF exam were performed: data obtained suggested motor neurone disease.
Informed consent was obtained before clinical and genetic investigation. Genomic DNA was isolated from peripheral blood leukocytes. All 17 exons and flanking intronic sequences of the SPG4 gene were amplified by PCR and directly sequenced on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems). The resulting sequences were compared to the sequences deposited in GenBank. The nucleotide change reported was checked in a panel of 100 healthy unrelated Italian subjects.

Results: Mutation analysis in the SPG4 gene allowed the identification in the patient's DNA of a heterozygous substitution in exon 1. The sequence variation was a G to A transition at position 194 (GenBank Acc. N°. AJ246001:c.194G>A) causing the substitution of arginine at position 65 with a histidine (p.R65H). This missense change is not described in the HGMD Mutation Database and was not found in a control population of 200 chromosomes. Moreover, the residue at position 65 is highly conserved among different species and *in silico* analysis using SIFT program (http://blocks.fhcrc.org/sift) revealed that the substitution is predicted to affect protein function with a score of 0.01 and therefore could cause loss-of-function or exert a dominant negative effect.

Discussion: The identification of this novel missense mutation in the SPG4 gene extends the spectrum of possible genotypes linked to an HSP phenotype and stresses the importance of screening the entire SPG4 gene in patients with a suspected motor neurone disease. These findings confirm the causative role of SPG4 haploinsufficiency in HSP and support genotype-phenotype studies performed so far, which have not revealed a clear correlation between type of mutation and age at onset or disease progression and severity and have suggested the existence of genetic modifiers modulating the phenotype.

P123 *DE NOVO* MUTATION IN THE SENATAXIN GENE IN A SPANISH PATIENT WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: senataxin, sporadic

Background: Aproximately 5–10% of cases of Amyotrophic Lateral Sclerosis are familial (FALS), and the remaining cases are sporadic (SALS). Mutations in several genes have been found associated to FALS including superoxide dismutase (SOD1,ALS1), alsin (ALS2), senataxin (SETX, ALS4), FUS-TLS (ALS6), dynactin (DCTIN1), angiogenin (ANG), synaptobrevin-associated membrane protein B (VAPB, ALS8) and TARDBP (ALS10) (1–4).
Most of the ALS cases with a known cause (approximately 20% of FALS and 5% of SALS) are due to SOD1 harboured mutations. Only the genes TARDBP and FUS-TLS, that account for 5% of FALS cases (3,4) are relevant, and the other ones account for a few kindreds. There have only been published mutations in three families with the SETX gene (2).

Objectives: To screen for mutations in the SETX gene in a subgroup of ALS patients, i.e. ALS with juvenile onset (younger than 35 years old) that harboured no SOD1 mutations.

Methods: Samples of 15 patients with probable or definitive ALS (12 SALS and 3 FALS) were screened for possible mutations in the SOD1 and SETX gene by genomic DNA sequencing using an ABI-PRISM 310 Genetic Analyzer (PE-Applied Biosystems).

The sequence variation found was confirmed by PCR-RFLP method (with AcuI, New England Biolabs) in the proband, other family members available and 200 controls.

Results: A new sequence variation in the SETX gene was found in one sample. This variation, not previously described, corresponded to a 1561_1562delCT that predicted a truncated protein: L521fsX527.
The patient developed weakness and muscle atrophy on the arms and dysartria. The neurological exam showed UMN and LMN signs in bulbar, upper and lower limbs.

Discussion and Conclusions: The first case, of ALS associated with SETX has been found in the Spanish population.
Senataxin encodes a protein with unknown function related not only to ALS but ataxia with oculomotor apraxia type 2 (AOA2). Only three missense mutations have been detected in patients with ALS4, while many mutations have been identified in patients with AOA2.
The patient fulfilled the El Escorial Criteria for the diagnosis of definite ALS with juvenile onset. None of the relatives of the patient in this study harboured the mutation, suggesting a *de novo* mutation in this sporadic patient.

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P124 A NOVEL LAMIN A/C (LAMIN A) G602S MUTATION IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: laminopathies, lipodystrophy, motor neuropathy

Background: Mutations in the lamin A/C gene (LMNA) are classified as either class I (cardiac complications, myopathies, neuropathies) or class II (partial lipodystrophy, progeria syndromes, mandibuloacral dysplasia). The occurrence of a LMNA mutation in association with sporadic ALS (sALS), cardiac dysrhythmia and lipodystrophy is unique.

Objectives: To describe a novel LMNA mutation ($LMNA^{G602S}$) in an individual with ALS, supraventricular tachycardia and lipodystrophy. The effect of this mutation on nuclear morphology *in vitro* will also be described.

Results: A 41 year old African American woman presented with a 6-year history of diffuse muscle cramping, hypertension, truncal obesity (BMI 35 kg/m^2), polydactyly and a supraventricular tachycardia requiring nodal ablation. Diffuse fasciculations, weakness and mild wasting of shoulder and intrinsic hand muscles, mild hip flexor weakness and hyporeflexia were observed. One year later, she developed diffuse weakness with bilateral foot drop and pathologically increased reflexes, consistent with a diagnosis of probable ALS. Electrophysiological studies demonstrated a multisegmental acute on chronic motor axonopathy with reduced motor response amplitudes and denervation on needle EMG. Based on the presence of a lipodystrophy with cardiac abnormalities, LMNA genomic sequence analysis was performed and a heterozygous $LMNA^{G602S}$ mutation was identified in the proband. Of the patient's 5 siblings, all female, two were found to have muscle cramping, lipodystrophy and hyporeflexia in association with the $LMNA^{602S}$ mutation. The father was clinically normal and genetically unaffected. The mother had died from breast cancer but was described as "looking like the index case". The $LMNA^{G602S}$ mutation was not found in 94 ALS cases from an archive of clinically and pathologically definite ALS (78 with sALS; 16 with ALS cognitive impairment (ALSci)). HEK 293T and Neuro2a cells were transfected with either EGFP-tagged wild-type (wt) LMNA or $LMNA^{G602S}$. The pattern of LMNA incorporation into the nuclear envelope was assessed by confocal microscopy at rest and following stress. Both wt-LMNA and $LMNA^{G602S}$ localized to the nuclear envelope in both a smooth and speckled pattern. An approximately 2-fold increase in speckled over smooth patterns was observed in wt-LMNA, while the two were equal in $LMNA^{G602S}$ transfected cells. Following stress, speckled morphology increased significantly in both, suggesting that at rest, $LMNA^{G602S}$ transfected cells were under stress.

Discussion and Conclusions: The $LMNA^{G602S}$ mutation has previously only been observed in a 24 year old female with hypoandrogenemia, polycystic ovarian syndrome, insulin resistance and acanthosis nigrans in the absence of lipodystrophy, cardiac abnormalities or a motor neuronopathy. The observation of a laminopathy in association with ALS is novel. The apparent heterogeneity of the clinical phenotype could be related to interactions with secondary genetic, epigenetic or environmental factors or mechanisms.
The presence of a motor neuropathy or ALS in association with cardiac conduction abnormalities or lipodystrophy should prompt a search for an underlying laminopathy.

P125 NEUROPATHY TARGET ESTERASE MUTATIONS IN APPARENTLY SPORADIC ALS

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Keywords: NTE, gene mutation, environment

Background: Neuropathy target esterase (NTE) is a widely distributed membrane phospholipase that is implicated in the pathogenesis of both organophosphorus compound induced delayed neuropathy (OPIDN) and autosomal recessive motor neuron disease (NTE-MND). In the case of OPIDN, a disorder that includes progressive spastic paraplegia and peripheral neuropathy, organophosphorylation of NTE's active site serine leads to inhibition of NTE esterase activity and formation of toxic or "aged" NTE. NTE-MND is an autosomal recessive MND characterized by progressive spastic paraplegia and wasting of distal upper and lower extremity muscles and is similar to that of OPIDN. Individuals with

NTE-MND have homozygous or compound heterozygous mutations involving NTE's esterase (NEST) domain.

Objectives: To investigate the possibility that abnormalities involving NTE or its biochemical pathways may contribute to other motor neuron disorders including amyotrophic lateral sclerosis (ALS) by examining the NTE coding sequence in subjects with ALS.

Methods: Analysis of the ten exons (exons 22 through 32) that together encode NTE's esterase domain was focused on because this was the location of NTE-MND mutations. Heteroduplex analysis and DNA sequencing were used to analyze NTE exons 22 through 32 in DNA samples from 100 individuals with apparently sporadic ALS and an equal number of control subjects.

Results: Three ALS subjects were found to be heterozygous for novel, amino acid substituting mutations that disrupted inter-species conserved residues in NTE's esterase domain: Q756R, G942A and H1023Y. One of these subjects had an additional heterozygous, novel, amino acid substituting mutation in an exon outside of NTE's esterase domain (V19A). Each of these mutations were absent in 100 control individuals and is predicted to be not tolerated by SIFT analysis. Analysis of the biochemical and functional consequences of NTE mutations in ALS subjects is in progress.

Discussion and Conclusions: These findings support the importance of NTE in motor neuron biology; and support the possibility that genetic variation in NTE is a contributing factor to the development of "apparently sporadic" ALS.

P126 CYTOKINE GENES AND AMYOTROPHIC LATERAL SCLEROSIS PREDISPOSITION

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Keywords: cytokines, polymorphism, lymphocytes

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder resulting in loss of spinal motor neurons. Levels of several proinflammatory cytokines have been found to be increased in patients, suggesting crucial implications of inflammation in the pathogenesis of ALS (1–3).

Objectives: To investigate at genomic level the distribution of 23 cytokine polymorphisms in ALS patients and controls. 23 functional polymorphisms of 12 different genes encoding for cytokines and receptors: IL-1α, IL-1β, IL-1R, IL-1RA, IL-4Rα, TGF-β, IL-12, IFNγ, TNFα, IL-2, IL-4, IL-6, IL-10 were genotyped. Allelic, genotypic and haplotype frequencies of the polymorphisms in these genes were assessed in 60 ALS patients and 140 healthy controls.

Methods: Cytokine polymorphism analysis was carried out by RFLP.

Results: These data showed statistically significant differences in CC/CT genotype distribution of IL-1β and CG/GG genotypes of TGF-β. Specifically, an increase in the frequency of the CC genotype in the IL-1 β gene ($P = 0.0347$) and GG genotype of TGF-β ($P = 0.013$). Moreover, there was a decrease in CT genotype frequencies in the IL-1β gene ($P = 0.0149$) and CG genotype in the TGF-β gene ($P = 0.0126$) in ALS patients compared to controls. Finally, these data showed a significant increase of AA genotype ($P = 0.019$) for −238 TNF polymorphism in patients compared to controls. Of note, there was a significant reduction of the TG haplotype (low producer) ($P = 0.0038$) of IL-2 cytokine and statistically significant increase of CC haplotype (low producer) ($P = 0.02279$) of TGF-β in ALS patients compared to controls.

Discussion and Conclusions: Studies on the role of TGF-β have suggested a potential role of this molecule in neuronal apoptosis. TNF-α is elicited in the brain following injury, neurodegeneration and neurotoxicity. Finally, IL-2 is a pleiotropic cytokine regulating Th1/Th2 balance and has a regulatory activity in brain function. These data point toward a potential role of genetic polymorphisms of inflammatory genes in the pathogenesis of ALS. IL-1β is involved in the regulation of inflammatory stress and central neurotransmission. These polymorphisms, if confirmed in a large panel of patients, will be useful as genetic markers of predisposition to ALS.

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P127 ANGIOGENIN LEVELS AND ANG GENOTYPES IN ALS

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Keywords: angiogenin, CSF, ANG

Background: Mutations in the ANG gene segregate with familial and sporadic ALS. To assess the contribution of ANG genotypes to angiogenin protein levels, five informative SNPs were genotyped across ANG in 661 Irish and Swedish patients with ALS and 580 matched controls. Additionally, angiogenin levels in plasma (110 cases, 144 controls) and CSF (102 cases, 100 controls) were quantified in the Swedish cohort.

Objectives: To investigate the relationship between CSF and plasma angiogenin levels in patients with ALS and to determine the contribution of genetic variants in the ANG gene to angiogenin expression.

Methods: Association analyses were performed using the computer programme PLINK with the following SNPs: rs9322855, rs8004382, rs4470055, rs17114699 and rs11701. Plasma and CSF angiogenin levels were quantified by enzyme-linked immunosorbent assay (ELISA). Contribution of individual SNPs to angiogenin levels was assessed using analysis of covariance (ANCOVA) and regression analyses.

Results: Of the five SNPs, rs17114699 showed significant association in both the Irish and Swedish cohorts ($P_{Irish} = 0.03$; $P_{Swedish} = 0.04$). Angiogenin levels were observed to vary in a graded, allele dose-dependent manner. Plasma

angiogenin levels were significantly different between cases and controls (P = 0.0002) whereas CSF angiogenin levels were not significantly different (P = 0.7). A correlation between plasma and CSF angiogenin levels was observed in controls that were absent in cases (P value for difference between linear regression lines, 0.0006).

Discussion and Conclusions: Angiogenin levels are dysregulated in patients with ALS. These studies have suggested that overall, the positive linear relationship between CSF and plasma angiogenin levels is perturbed in cases. This suggests a tissue-specific downregulation of angiogenin in ALS. Recent reports have suggested that such observations can be due to the altered activity of microRNAs (miRNAs). These are small endogenous noncoding RNAs that can affect gene silencing at the posttranscriptional level. The ANG gene contains several predicted miRNA binding sites. Current work is focussed on assessing the contribution of risk haplotypes to the efficiency of miRNA-mediated silencing of the ANG gene.

P128 ApoEε4 ALLELE IS RELATED TO A WORSE OUTCOME IN ITALIAN ALS PATIENTS

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Keywords: angiogenin, CSF, ANG

Background: Many studies have looked for an interaction between Apolipoprotein E (present in 3 isoforms ε2, ε3 and ε4) and neurological disorders. In particular, an association between the allele ε4 of ApoE and Alzheimer disease (AD) has been widely recognized. The ApoEε4 represents an important risk factor in the development of AD and also reduces the age of onset.

Objectives: To study the possible association of the ApoEε4 and ALS and clinical characteristics in a series of Italian ALS patients.

Methods: Blood samples of 116 ALS patients (10 familial ALS and 106 sporadic ALS) and of 118 controls (healthy volunteers) were collected, between January 2000 and March 2001.

Results: The frequency of the allele in cases and controls was similar. The mean age of onset of patients with at least one E4 allele was not different from that of patients without ε4 alleles (58.9 (SD 12.2) vs. 57.3 (SD 13.7)). The presence of an ε4 allele did not influence the site of onset.
But the median survival of ALS patients with the ε4 allele was 26.0 months, significantly lower than that of patients with ε3/ε3 and ε2/ε3 (34.6 months) (P = 0.025). In particular, the presence of at least one ε4 allele increased the risk of death by 2.04.

Conclusions: An association has been found between the presence of ApoEε4 and ALS in a series of Italian ALS patients: the ε4 allele has a negative influence on the course of the disorder and it increased the risk of death.

P129 SPORADIC CASE OF INCLUSION BODY MYOSITIS-PAGET DISEASE AND FRONTOTEMPORAL DEMENTIA MIMICKING AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: IBM, FTD, VCP

Background: Hereditary inclusion-body myositis associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) is a rare autosomal-dominant disorder caused by mutations of the valosin-containing protein gene (VCP). The clinical phenotype is variable and the majority of patients are erroneously diagnosed with limb girdle muscle dystrophy, facioscapular muscular dystrophy, spinal muscular atrophy or even amyotrophic lateral sclerosis (ALS).

Objectives: To present the case of a patient with clinical features mimicking sporadic ALS-Fronto-temporal dementia (FTD) and who was finally diagnosed with IBMPFD.

Methods: The clinical phenotype of this patient including age of onset, initial symptoms and neurological findings during the course of the disease including neuropsychological tests were described. The results of biological tests including needle electromyography, bone radiographies, SPECT and genetic testing were also recorded.

Results: The patient is a 79-year-old lady who was seen in the outpatient clinic in order to perform a clinical screening of ALS-FTD. She had already been diagnosed with FTD three years earlier because of progressive behavior, desinhibition and gambling problems. In the last year she complained of walking difficulties secondary to lower limb weakness and clumsiness of both hands. Neurological examination showed distal atrophy of hands and legs with asymmetric proximal and distal weakness of lower limbs that was more noticeable in the left side. She had normal osteotendinous reflexes in the upper limbs but brisk in lower limbs. Sensory examination was normal.
Electromyographic tests showed a neurogenic pattern in hands and lower limbs suggesting a possible motor neuron disease.
The biological tests showed high level of serum Alkaline Phosphatase (ALP) leading to the suspicion of Paget disease.This diagnosis was confirmed by skeletal radiographies.
The combination of FTD, Paget disease and neuromuscular disorders led us to search for a mutation in the VCP gene despite the absence of family history and the neurogenic pattern in EMG. The results showed a c.283C > T (p.Arg95Cys) mutation in the VCP gene.

Discussion: As recently shown by Kimonis *et al* in a review of a series of 49 patients from 9 different families, the clinical features of IBMPFTD are heterogenous. Muscle disease was present in 87% of cases but it was erroneously diagnosed in the majority of them as limb girdle muscle dystrophy, fascioscapular muscular dystrophy, late onset distal myopathy or even ALS.
This patient is the first sporadic case reported with IBMPFD, its late onset supporting the clinical heterogeneity of the disease. This entity should also be suspected in elderly patients even in the absence of family history when there is

an association of FTD, limb weakness of variable topography and high levels of serum ALP.

P130 IDENTIFICATION OF GENES REGULATED BY TDP-43 REVEALS A GLOBAL ROLE IN RNA PROCESSING AND CELL DEATH PATHWAYS

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Keywords: TDP-43, microarray, RNA processing

Background: Ubiquitinated cytoplasmic inclusions containing TDP-43 are a pathological hallmark of ALS. Mutations in the TDP-43 gene have been found in both familial and sporadic ALS cases. TDP-43 is an RNA/DNA binding protein involved in gene regulation through control of RNA transcription, splicing and transport. The function of TDP-43 in the nervous system is currently unknown and its role in the pathogenesis of ALS is unclear. TDP-43 binds to $(UG)_n$ repeats in target RNA's, facilitating mRNA splicing and transport. A few RNA targets have been reported in literature, but given the abundance of $(UG)_n$ repeats in the genome many more targets are likely to exist.

Objectives: To identify genes in the central nervous system that are regulated by TDP-43.

Methods: RNA-immunoprecipitation followed by microarray analysis (RIP-CHIP) was used to isolate and identify RNA bound to TDP-43 protein. Following lysis of mouse brain tissue, protein was immunoprecipitated with anti-TDP43 antibody; RNA was then isolated and hybridized to Affymetrix mouse expression chips. Microarray data was analysed using Partek Genomics Suite to identify genes, followed by Ingenuity Pathway Analysis to identify networks.

Results: Partek analysis of immunoprecipitated RNA produced a list of 2,910 potential TDP-43 gene targets. The list included three ALS related genes (ALS2, VEGFA and EAAT2), implying the mRNA's produced from these genes are direct targets of TDP-43. Pathway analysis found multiple genes were related to RNA post-transcriptional modification and apoptosis.

Discussion and Conclusions: This is the first study to combine RNA immunoprecipitation with microarray chip analysis (RIP-on-chip) for the identification of TDP-43 targets. Known ALS genes are direct targets of TDP-43, providing a possible link between familial and sporadic cases. Multiple genes are related to RNA post-transcriptional modification, a known function of TDP-43. Aberrant RNA processing could be important in the pathophysiology of ALS and has been previously implicated as a cause of loss of EAAT2 in ALS patients. Pathway analysis revealed that RNA targets of TDP-43 are also associated with apoptosis, implying TDP-43 has a direct role in motor neuron cell death. Aggregation of TDP-43 in cells of ALS patients may not be pathogenic itself, but may prevent TDP-43 performing its normal cellular functions. These studies have the potential to improve the understanding of the molecular mechanisms involved in ALS and provide rational targets for new therapies.

P131 GENETIC SCREEN AND FUNCTIONAL ANALYSIS OF TARDBP MUTATIONS

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Keywords: genetics, TDP-43, splicing

Background: Mutations in TARDBP, the gene encoding the TAR-DNA binding protein (TDP-43), have recently been identified in patients with both sporadic and familial amyotrophic lateral sclerosis (ALS), but the pathogenic effects of mutant protein are largely unknown.

Objectives: To determine the prevalence of TARDBP mutations in a specialist clinic population in Oxfordshire and functionally model the effect of mutations in cell culture systems.

Methods: Direct sequencing of TARDBP and real-time PCR were used for copy number variation. Identified variants were cloned and examined in cell culture for sub-cellular localisation differences. In addition, a splicing reporter minigene was employed to assess whether mutations had an impact on the protein's splicing inhibitory function.

Results: Three TARDBP variants in a total of 136 patients with ALS were identified. The variants included two previously reported synonymous changes (A66A and A315A) as well as a novel protein changing mutation, A321G, in a patient with sporadic ALS. The A321G mutation is located in the C-terminal domain of TDP-43 in close vicinity to most other known TARDBP mutations. A C-terminal deletion construct displayed altered nuclear and cytosolic distribution and lack of splicing inhibitory function in a trans-acting splicing assay. The A321G mutation, like several other described mutations, however, did not induce distinguishable abnormalities in cell culture assays compared to wild-type TDP-43 with regards to cell survival, splicing inhibition or stress response.
Functional analysis using a splicing reporter minigene ruled out a significant cis-acting effect of the synonymous changes on splicing of the TARDBP pre-mRNA.

Conclusions: These data give further evidence of the genetic contribution of TARDBP mutations to sporadic ALS, while the lack of obvious abnormalities of mutant protein *in vitro* underlines the complexity of disease pathophysiology.

P132 TARDBP GENE ANALYSIS IN ALS PATIENTS

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Keywords: TDP-43, mutation, TARDBP

Background: Increasing evidence suggests a direct role of the TAR DNA-binding protein 43 (TDP-43) in neurodegeneration (1). Several point mutations in the TARDBP gene, coding for TDP-43, have been reported in familial and sporadic amyotrophic lateral sclerosis (ALS) cases (2).

Objectives and Methods: To contribute to the estimation of the frequency of TARDBP gene mutations in ALS, by performing a TARDBP gene sequence analysis in a cohort of 80 ALS patients, (71 sporadic and 9 familial). DNA samples obtained from the peripheral blood of each patient were studied by Single Strand Conformation Polymorphism (SSCP) analysis and direct sequencing of resulting conformers.

Results: This analysis identified a previously reported heterozygous missense c.1144G>A (p.A382T) mutation in two unrelated ALS patients of the cohort. The first patient was a 50-year-old Northern Italian man who presented with a typical ALS phenotype, with disease onset in the spinal region at the age of 50 years. He had a family history suggestive of autosomal dominant inheritance: his father was diagnosed as affected by ALS at 50 years. The same mutation in TARDBP gene was identified in the DNA from his son and his daughter (respectively, 22 year- and 27-year old) and his 45-year-old sister, all healthy at the time of this study. The second ALS patient carrying the TARDBP c.1144G>A mutation was a 38-year-old Southern Italian man presenting with a left spastic hemiparesis, widespread fasciculations, Hoffman sign and plantar extensor response present bilaterally and difficulties of speech and swallowing. Diffuse bradykinesia, dystonic contraction of the platysma and the lower facial musculature on the left, a resting tremor of the head, facial hypomimia and infrequent blinking were also present. Furthermore, the patient showed Parkinson-like micrographia, anosodiaphoria and bradyphrenia. The patient's parents at age 68 and 65 and the 36 year-old brother were healthy. DNA from these family members were unavailable.

Discussion and Conclusions: These findings confirmed that TARDBP gene mutations are not a frequent cause of sporadic ALS (1.4%). However, they appeared to be a relevant cause of familial ALS (11.1%). The identification of a TARDBP gene mutation in an ALS patient with additional extrapyramidal features suggests that mutated TDP-43 may exert a pathological effect in brain regions associated with extrapyramidal functions of ALS patients.

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P133 MUTATION OF TDP-43 GENE IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN SOUTH-WEST CHINA

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Keywords: TDP-43 gene, mutation

Background: Amyotrophic lateral sclerosis (ALS) is one of the most common fatal neurodegenerative disorders.

Objectives: To investigate the mutation frequency of TDP-43 gene in patients with sporadic ALS in South-West China.

Methods: All the patients included in this study were diagnosed with ALS according to the El Escorial criteria from May 2004 to January 2009. Data of clinical features and outcome of patients were registered. Blood samples were collected after informed consent. All the exons of TDP-43 gene were amplified by PCR and sequencing.

Results: Overall 120 patients diagnosed with definite and probable ALS were included in this genetic study. Two mutations were found including p.378N>S and p.572G>A, R191K.

Conclusions: These findings suggest that mutation of the TDP-43 gene is not rare in Chinese patients with SALS.

P134 MUTATIONS IN FUS CAUSE FALS AND SALS IN FRENCH AND FRENCH CANADIAN POPULATIONS

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Keywords: FUS, mutations, sporadic cases

Background: Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of motor neurons in the brainstem, motor cortex and spinal cord; this degeneration manifests itself through upper and/or lower motor neuron dysfunction. The disease is characterized by muscle weakness and atrophy relentlessly spreading to other parts of the body. Death is usually due to respiratory failure. The profile of genes mutated in ALS has gradually expanded over the past two years. The primary causative gene remains the zinc copper superoxide dismutase gene (SOD1) in which mutations account for ~15–20% of familial ALS (FALS) cases and which altogether represents ~2% of the combined sporadic (SALS) and FALS case.
However, several mutations were recently reported in the TAR-DNA binding protein (TARDBP) gene encoding the TDP-43 protein at the ALS10 locus. This mutation search was initiated following analysis revealing that TDP-43 is a major constituent of the neuronal aggregates observed in both ALS and frontotemporal dementia (FTD) patients. The identification of mutations in TARDBP led other teams investigating the ALS6 locus on chromosome 16 to consider the FUS gene as an ideal candidate because the two proteins share RNA processing functions. The identification of a homozygous mutation in a recessive family with ALS from Cape Verde and of heterozygous changes in dominant ALS pedigrees led to the conclusion that FUS was indeed the causative gene.

Objectives: To validate the results recently obtained on chromosome 16 by sequencing the FUS gene in a panel of FALS and SALS cases.

Methods: The entire coding region of the FUS gene was screened in a cohort of 200 ALS patients. An additional 190 patients with sporadic ALS were screened for variants in exon 15 for which mutations were previously reported.

Results: In total, three different mutations were identified in four different patients, including one 3 bp deletion in exon 3 of a SALS patient and two missense mutations in exon 15 of two SALS and one FALS patients.

Discussion: The overall percentage of mutations identified in this study was 1.25% in FALS (1/80) and 2.5% in SALS (3/120) cases. This is less than what was originally reported. Also, this study only detected heterozygous changes while the first reports described both homozygous and heterozygous changes. The identification of mutations in the FUS gene is providing the field with new insights about the mechanisms involved in ALS and helps to develop a more comprehensive picture of the genetic events underlying this neurodegenerative disease.

Conclusions: This study identified sporadic patients with mutations in the FUS gene. Two mutations which were previously described in FALS cases were also identified in two SALS patients. One new deletion was also identified in a SALS individual.

P135 CLINICOPATHOLOGICAL CHARACTERIZATION OF FUS MUTATIONS IN A PROSPECTIVE ALS COHORT

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Keywords: TDP-43, genetics, frontotemporal syndromes

Background: Chromosome 16 linked familial ALS (fALS) has recently been shown to be caused by mutations in the FUS/TLS gene (FUS). The exact frequency of FUS mutations and the full spectrum of clinicopathological correlates remains to be determined.

Objectives: To determine the frequency of FUS mutations in a well-defined prospectively characterized cohort of ALS patients and define its clinicopathological features.

Methods: Mutation analyses of FUS were performed by sequencing all 15 FUS exons in archival tissues (1990–2008) from the London Motor Neuron Disease clinic. This included 94 cases of clinically and neuropathologically definite ALS, of which 16 had ALS with cognitive impairment (ALSci). Seven families were included (total of 9 cases).

Results: A 46 year old fALS patient with limb-onset ALS of 2 years duration, without cognitive impairment, was found to have a missense mutation (c.1561C > T; p.R521C) in FUS. Post mortem spinal cord tissue demonstrated a severe loss of anterior horn cells. FUS-immunoreactive (FUS-ir) neuronal cytoplasmic inclusions (NCI) included large, round dense aggregates and collections of fine granules and filaments. FUS-ir dystrophic neurites were observed as were moderate numbers of FUS-ir glial inclusions in the grey matter and in white matter fasciculi. No TDP-43 immunoreactive (TDP-43 ir) inclusions were observed, however, confocal microscopy demonstrated a diffuse increase in cytosolic TDP-43 immunoreactivity, typical of the response to axonal injury. Two additional novel FUS variants were identified in the series. These included a silent mutation (c.1566G > A; p.R522R) and a change in the 3'UTR (c.*41G > A) that was also present in 4 out of 221 control individuals (1.8%). Both of these cases showed TDP-43-ir inclusions and an absence of FUS-ir pathology, suggesting that these FUS variants are non-pathogenic.

Discussion: A single pathological FUS mutation (p.R521C) has been observed amongst 94 clinically and neuropathologically confirmed ALS cases.

Conclusions: A single pathogenic FUS mutation was identified in 7 (14%) fALS cases and none of the 85 sALS cases in a clinically and neuropathologically well-characterized cohort of ALS patients.

P136 ASSOCIATION STUDY OF THE SNPS IN GSTA1, GSTO2 AND NQO1 GENES WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

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Keywords: GST, NQO1, SNP

Background: Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disorder. Approximately 90% of cases are sporadic (SALS), the others are familial cases (FALS). Oxidative stress is considered one of the mechanisms involved in ALS pathogenesis. Activation of the Nrf2/ARE signaling pathway can up-regulate a series of antioxidant enzymes, including glutathione S-transferases (GSTs) and NADPH quinone oxidoreductase 1 (NQO1). GSTA1 and GSTO2 are two newly identified subgroups of GSTs. The polymorphisms of GSTA1, GSTO2 and NQO1 might result in decrease or loss of their activities.

Objectives: To investigate the association between polymorphisms of GSTA1, GSTO2 and NQO1 and the risk of ALS.

Methods: The case-control study included 143 SALS patients and 210 healthy controls. Venous blood from each subject was drawn and genomic DNA was extracted. Single nucleotide polymorphisms (SNPs) of GSTA1, GSTO2 and NQO1 were genotyped by polymerase chain reaction-restrictive fragment length polymorphism (PCR-RFLP) analysis.

Results: All genotyped SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$). The SNPs of GSTA1 C69T and NQO1 C609T had no association with susceptibility to SALS. However, the risk of ALS was significantly increased in subjects with GSTO2 N142D ND genotype, especially GSTO2 N142D DD genotype. (OR = 2.12, 95% CI = 1.36 ~ 3.32; OR = 3.30, 95% CI = 1.04 ~ 10.45, respectively). Therefore, ND and DD genotypes of GSTO2 N142D may be potential risk factors for SALS.

Discussion: Recent studies found that Nrf2-ARE activation was a novel neuroprotective pathway. Genetic polymorphisms of GSTs and NQO1 have important implications for drug efficacy and cancer susceptibility, but the association of SNPs in the GSTA1, GSTO2 and NQO1 genes with susceptibility to ALS has been scarcely studied. Recently, significant association of Alzheimer's disease with NQO1, GSTO1 and 2 genes had been identified, and another study suggests a possible effect of the GSTO1 and 2 loci on age

of onset of FALS. Similarly, this study found that GSTO2 gene was associated with ALS. However, the association of ALS with GSTA1 and NQO1 genes was not found. Different genetic backgrounds and environments might have led to the distributional difference of GSTA1 and NQO1 among various races and areas. Furthermore, this polymorphism might not be an independent risk factor for ALS. Further prospective investigations using large populations among various ethnic groups are needed to determine the general applicability.

Conclusions: These findings indicate that genetic polymorphism in the GSTO2 gene are associated with ALS in China.

P137 CLINICAL AND GENETIC FEATURES OF PATIENTS WITH FAMILIAL ALS: ANALYSIS FROM A CHINESE DATABASE

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Keywords: SOD1, TARDBP, FUS/TLS

Background and Objectives: Mutations in the SOD1 gene are found in approximately 20% of familial ALS (FALS), while TARDBP and FUS/TLS genes are ~3% and ~4%, respectively. Few data of FALS in China is available to date. This study aimed to report these gene mutations in Chinese patients based on a country-wide database.

Methods: From 2005 to 2009, the cohort consisted of 1,200 ALS patients, including 33 FALS families. The geographic distribution of the patients covered >93% of Chinese administrative provinces (except for Tibet and Taiwan). 20 of 33 families have been screened for SOD1, TARDBP and FUS/TLS gene mutations.

Results: Thirty-three families came from 14 provinces, with bulbar-onset accounting for 6% and the rest had limb-onset. 16 families included 2~4 affected individuals (49%), while 17 had >5 affected individuals (51%) and 7 pedigrees had >10 in 4~6 generations (21%). The total patients from these 33 pedigrees were 211 (17.5%) and the male-to-female ratio was 50.2%, with a median age of onset of 46.6, from 20 to 67. The average duration from the symptom onset to diagnosis was 44.7 months. Following their diagnosis, a median lifespan was 6.9 years, from only 1 year to beyond 20 years. Most patients had an autosomal dominant inheritance (94%) and two families had an autosomal recessive inheritance.
SOD1 analysis was performed in 20 of 33 FALS pedigrees and 7 different missense SOD1 mutations in 7 families (21%) were identified (H46R, A4S, G16A, E100G, G41D, S105L and G133V), in which G133V was a novel mutation. TARDBP gene was sequenced in 13 SOD1-negative FALS patients and 2 different missense mutations (one known p.S393L and a novel p.M336V) in exon 6 were indentified in 2 kindreds with an autosomal dominant inheritance. The novel mutation p.M336V was identified in all 18 patients in a large F8 family with 6 generations, in which bulbar- and limb-onset coexist in the pedigree. The mutation p.S393L was observed in 2 limb-onset patients in F11 family. The frequency of TARDBP gene mutations were 10% (2/20). The FUS/TLS gene was also sequenced in SOD1 and TARDBP gene-negative patients. Three mutations in exon 15 in 3 indexes of different pedigrees were detected. All probands had limb-onset. One was male with onset at the age of 20; the two others were female with onset at 48 and 49, respectively. Further analysis on these FUS/TLS gene mutation is ongoing. The frequency of the gene mutations was 15% (3/20).

Conclusions: The percentage of Chinese FALS was 17.5%, which was higher than that reported in literature. The frequency of gene mutations of SOD1 (21%), TARDBP (10%) and FUS/TLS (15%) were also higher. The survival of FALS cases seemed longer, but the bulbar-onset and the male-to-female ratio were lower than reported.

P138 A NOVEL SOD1 MUTATION (P69S) WITH LOW PENETRANCE IN FAMILIAL ALS

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Keywords: familial ALS, SOD1, P69S

Background: To date, 135 SOD1 gene mutations have been reported. Clinical courses are quite variable both with mutations among different exons and within the same exons. Disease severity, with respect to age at onset and disease duration, also varies within and between families. In most familial ALS patients, inheritance is autosomal dominant, although some cases have been reported by autosomal recessive inheritance. Penetrance is incomplete and complicated by age dependence. However, previous studies have suggested that the phenotypes resulting from SOD1 gene mutations can be influenced by other factors, such as the environment and sex hormones, as well as the type of SOD1 mutation.

Objectives: To observe a family with a novel mutation in the SOD1 gene and report clinical characteristics with genetic analysis.

Methods: A 44-year-old woman presented with a five year history of progressive leg weakness followed by arm weakness. To evaluate the presence of a SOD1 mutation, DNA sequencing was performed. After the confirmation of SOD1 mutation in the proband, her clinical history was noted and genetic studies were performed on the proband's family members.

Results: Direct sequencing analysis of the SOD1 gene in the proband revealed a heterozygous C to T transition at cDNA position 205, which converted proline to serine at amino acid position 69 (P69S). This mutation has not been reported previously and was not observed in 100 control chromosomes. Therefore, further DNA analysis of several family members was performed and revealed that her mother, younger brother and son had the same mutation. The family had unique clinical features. The proband was the only affected member while the three others were asymptomatic and presumably unaffected. Her mother had yet to develop symptoms even after reaching the age of 65 years.

Discussion: The proband was the only affected person despite the presence of the mutation in the other three family members. This novel mutation may be associated with a relatively low penetrance. Investigations into environmental and genetic modifying factors are essential to future research.

P139 AN ITALIAN CASE OF SPORADIC ALS ASSOCIATED WITH ATYPICAL CLINICAL PRESENTATION OF THE HOMOZYGOUS D90A SOD-1 MUTATION

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Keywords: D90A, SOD1 mutation, clinical phenotype

Background: The homozygous D90A Cu/Zn superoxide dismutase 1 (SOD-1) gene mutation, first reported in 1995 and mainly affecting patients of Scandinavian origin, has also been detected in the Mediterranean population. The phenotype linked to this mutation is uniform and characterized by slowly progressive ascending paresis of both lower and upper motor neurones. The mean age of the onset of the disease is 42 ± 4.4 years and the mean survival is 11 ± 1.3 years. The high D90A allele frequency in the Scandinavian population and the homogeneous phenotype of the clinical presentation of the disease are presumably related to Viking migration and to a common specific founder.

Methods: The case study is a 67-year-old Italian male carrying the homozygous D90A mutation in the SOD-1 gene with one-year disease history beginning in his mid sixties with sporadic diffuse limb fasciculations, muscle cramps, weakness and wasting prevalently in his left limbs. Cognitive, bulbar, respiratory and cerebellar functions, as well as eye movements, sphincter function and sensory functions were intact. Spinal cord and brain MRI results were normal, as well as blood tests and CSF analysis. EMG showed neurogenic changes with denervation activity at rest in the cervical, thoracic, and lumbo-sacral regions. Motor-evoked potentials by transcranial magnetic stimulation indicated bilateral upper motorneuron involvement. Both muscle and sural biopsy have been performed in a different center, with detection of chronic neurogenic damage and muscle axonal neuropathy. Currently his ALS-FR scale total score is 42.

Conclusion: Therefore, this patient presented with a different clinical phenotype: the age of onset was 65 years, with initial involvements of the arms, absence of sphincter dysfunction and with an apparent aggressive course. This case supports the hypothesis that ALS phenotype linked to D90A mutation might be more heterogeneous than expected, at least in the Mediterranean population.

P140 A NOVEL SOD1 MUTATION IN A YOUNG ALS PATIENT ASSOCIATED WITH SLOWLY PROGRESSIVE CLINICAL COURSE

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Keywords: SOD1, novel mutation, slowly progressive clinical course

Background: Approximately 10% of ALS cases are familial and the mutation in the Cu/Zn superoxide dimutase (SOD-1) accounts for 20% of them. More than one hundred SOD1 mutations have been described, sometimes carrying peculiar phenotypes. Moreover, mutations in the SOD1 gene have been described in "apparently" sporadic ALS cases. In this study, a sporadic ALS patient with very slowly progressive clinical course who has a novel SOD1 mutation has been identified.

Methods: In 2000, a 40 year-old man with no family history of ALS, developed unilateral weakness of the distal left leg muscles. Neurological examination showed lower motor neuron signs with atrophy mainly in the lower limbs, but also to the intrinsic hand muscles. He had fasciculations in all four limbs. Tendon reflexes were brisk. No bulbar signs were detected. Sensation was normal. Brain and spine MRI were normal. Extensive screening for motor neuropathy was negative. Genetic testing for hereditary neuropathies, spinal muscular atrophy and Kennedy's disease were negative. Nerve conduction studies were normal. EMG revealed acute and chronic denervation changes in the muscles of the four limbs and in the bulbar region. No response to intravenous human immunoglobulins or to immunosuppressive drugs was observed. A diagnosis of motor neuron disease was made. In the following years, the disease slowly progressed, but even after 9 years the patient did not require measures of vital support. The father died at 75 years of age from heart disease. In 2008, at the age of 76, his mother was diagnosed with Lewy Body Disease. At the age of 49, the neurological examination of the patient showed severe weakness and atrophy in the four limb muscles especially in the distal districts, reduced tendon reflexes and fasciculations to the lower limbs, mild tongue atrophy with fasciculation and mild dysphonia. DNA samples were obtained and the five exons of the SOD1 gene were amplified by PCR. Sequence analysis identified a heterozygous transition resulting in aspartate to tyrosine aminoacid substitution at codon 11 of the SOD1 protein.

Discussion: This case may expand the phenotypic spectrum of sporadic ALS associated with SOD1 mutation. The mutation identified in this patient has not been reported previously and might be associated with the slow progression of ALS. Other SOD1 mutations produce a slowly progressive phenotype (G37R, G41D, H46R and E100K) which may be explained by a less important functional role of the affected aminoacid residue within the SOD1 protein. Analysis of the DNA of the patient's mother and sister is now in progress to understand the role of this mutation on patient's and mother's neurodegenerative diseases.

P141 DETECTION OF DIFFERENTIAL GENE EXPRESSION AND ALTERNATIVE SPLICING INDUCED BY MITOCHONDRIAL DYSFUNCTION USING WHOLE-GENOME EXON MICROARRAYS

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Keywords: gene expression regulation, alternative splicing, mitochondrial insufficiency

Background: Amyotrophic Lateral Sclerosis (ALS) is a complex neurodegenerative disease selectively affecting motor neurons. The causes of this disorder are heterogeneous, however pathogenesis is partially understood in familial ALS (fALS) where some cases are caused by mutations in Cu/Zn superoxide dismutase 1 (SOD1), a powerful antioxidant that protects the body from damage caused by superoxide. Mitochondrial dysfunction, protein misfolding, axonal transport disruption, among others have been suggested as

ALS causes. Interestingly aberrant mRNA alternative splicing has also been observed in ALS patients.

Objectives: To identify alterations in (i) gene transcription and of (ii) pre-mRNA alternative splicing (AS) induced by mitochondrial dysfunction.

Methods: In order to define the molecular mechanisms underlying the response to mitochondrial insufficiency, whole-genome analysis was performed on two cellular models of neurodegeneration: SH-SY5Y neuroblastoma cells 1) untreated, or treated with paraquat (PQ), a toxic herbicide that impairs mitochondrial function and 2) stably expressing either wild type SOD1 or a mutant SOD1 protein carrying the G93A fALS mutation. Cells were profiled with Exon 1.0 ST GeneChips (Affymetrix), which allow the definition of both transcription patterns and alternative pre-mRNA maturation events. The data set from the two experiments were then combined in order to discover genes whose expression was significantly altered in both sets and showed a common trend. The identified genes were analyzed for their functions and involved pathways using the Ingenuity Pathways Analysis (IPA7) software.

Results: At the transcription level, about 160 genes showed altered expression in both PQ-treated and mutant SOD1 cells. Pathway analysis identified "cell cycle" and "nervous system development and function" as top affected networks. Interestingly, 28 genes are associated to neurological disorders, 2 to glycosphingolipid biosynthesis and to the glycerolipid metabolism. At the pre-mRNA splicing level, 38 genes showed altered AS. Pathway analysis of these genes identified "Genetics Disorders" and "Neurological Diseases" as common categories.

Discussion and Conclusions: This study analyzed, on a genome-wide scale, the cellular response to mitochondrial failure in two cellular models of neurodegeneration with the aim of identifying common dysregulated pathways. The analysis of the transcription profile and of the alternative splicing pattern allowed the identification of a small subset of genes linked to neurological disorders and cell cycle control. Experimental validation of the microarray results is currently underway. Next, functional activities of the identified candidate genes and of particular protein isoforms generated by alternative splicing events will be investigated to define their role in ALS pathogenesis.

P142 QUANTITATIVE TRAIT LOCI (QTL) LINKED TO PHENOTYPE IN THE G93A SOD1 TG MOUSE

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Keywords: QTL, modifier, genetic background

Background: The genetic heterogeneity described in familial ALS kindreds suggests the existence of genetic modifiers. It has previously been reported that phenotype is dependent on genetic background in mice that carry the human G93A SOD1 transgene (Tg). When the Tg is bred onto the C57BL/6 (B6) background, survival is prolonged compared to survival of mice with the Tg bred onto the SJL background (B6: 143.6 $\pm$ 7.5 days vs SJL: 119.2 $\pm$ 9.7 days), further supporting the presence of genetic modifiers.

Objectives: To use these congenic lines to identify genetic loci that are linked to the phenotype variability.

Methods: B6 and SJL congenic lines of hSOD1Tg (G93A) were backcrossed to create two F2 intercrosses totaling 188 animals. Survival was recorded for each F2 Tg mouse and tail DNA was sent to Jackson Laboratory for haplotype analysis. A whole genome scan was performed using 128 pseudo markers covering the mouse genome. Pair wise scans were performed using 2 cM spacing. All possible pairs of Quantitative Trait Loci (QTL) locations on each chromosome were tested for association with the life span. The likelihood from the full model (pseudo-marker pair and the interaction between them) and the null model (no genetic effect) was compared and LOD scores were calculated. QTL and the possible QTL-QTL interaction identified from a single QTL scan and pair wise scan were fit into multiple regression models. P values for terms in the multiple regression model were calculated.

Results: The linkage data demonstrated a LOD score of 6.26 in the full model (age at death = sex + QTL + sex*QTL) and 6.18 in the additive model (age at death = Sex + QTL) for a QTL on Chr 17 located at 5.47 cM (confidence interval 5.47–35.5 cM). Animals carrying BB alleles at the Chr 17 QTL lived a mean of 146.9 $\pm$ 15.4 days while those with SS alleles lived 126.7 $\pm$ 10.9 days. A QTL on Chr 7 had a LOD score of 3.08 (full model) and 2.36 (additive mode) at a locus located at 49 cM (confidence interval 16–84.58 cM). Animals with SS alleles on Chr 7 also had shorter lifespans. Multiple regression analysis showed no linkage to gender. The Chr 17 locus has also been identified at Jackson Laboratory using a different strain combination, through this collaboration.

Discussion and Conclusions: Chromosomal regions have been identified on mouse chromosome 17 and 7 that link to survival in G93A transgenic mice. Further studies and fine mapping of the linked regions will enable the identification of genes that potentially modify the phenotype of motor neuron disease in the mouse model. Understanding the role of such modifying genes may lead to a better understanding of the pathogenesis of ALS and provide new therapeutic targets.

P143 TAU AND ITS ROLE IN HUMAN ALS AND MOTOR NEURON DEGENERATION IN THE SOD1G93A MOUSE MODEL

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Keywords: H1/H2 polymorphism, MAPT, SOD1 G93A

Background: Neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) are characterized by axonal dysfunction. Microtubules and their associated proteins, as well as neurofilaments, are the main components of axonal architecture and have been hypothesized to contribute to the pathogenic mechanism of motor neuron degeneration in ALS. Aberrant phosphorylation of the microtubule-associated protein tau has been found in the brain tissue of ALS patients and in the spinal cord of the mutant SOD1 mouse, a rodent model for ALS. In addition, a possible, but not conclusive genetic

association between polymorphisms in the tau-encoding *MAPT* gene and the occurrence of ALS has been reported.

Objectives: To investigate the possible association of the H1/H2 polymorphism in *MAPT* with ALS by performing a genetic association study. To further elucidate the biological role of tau in ALS by studying the effect of lowering tau expression levels in the $SOD1^{G93A}$ mouse.

Methods: A total of 3,594 well-characterized ALS patients and 9,400 controls from seven cohorts were genotyped for the *MAPT* inversion polymorphism. This H1/H2 polymorphism was tagged by SNP rs9468 or by SNP rs8070723.
The $SOD1^{G93A}$ mouse was crossed with a mouse in which the *MAPT* gene has been deleted ($MAPT^{-/-}$). Motor neuron degeneration was monitored by determining disease onset (failure of motor performance on rotarod) and survival. Motor neuron loss was quantified by counting and determining the area of neurons in the lumbar spinal cord of symptomatic mice.

Results: Despite the well-powered study population, no association was observed in a combined analysis (H1/H1: OR = 1.07, 95% CI = 0.98–1.17, P = 0.115). Lower tau levels in transgenic $SOD1^{G93A}$ mice increased survival of these mice at a non statistically significant level (139 ± 12 days for $SOD1^{G93A}/MAPT^{+/+}$ mice, 148 ± 10 days for $SOD1^{G93A}/MAPT^{+/-}$ mice and 145 ± 14 days for $SOD1^{G93A}/MAPT^{-/-}$ mice; n = 18, 19 and 15 respectively; P = 0.2), without changing age of onset of the clinical motor deficits. To exclude a subtle effect on motor neuron survival that would escape behavioural or survival analyses, the number of motor neurons in the spinal cord in $SOD1^{G93A}$ and double transgenic mice was quantified, but only the lack of effect of partial or complete tau deletion was confirmed.

Discussion and Conclusions: These results demonstrate that tau does not contribute to the pathogenesis of sporadic ALS in humans and of mutant SOD1-induced motor neuron degeneration in mice, in contrast to what has been found in other neurodegenerative diseases such as progressive supranuclear palsy (PSP) and Parkinson's disease. Therefore, reducing tau protein levels is unlikely to present a therapeutic strategy for ALS.

P144 A SOD1 MUTATION ASSOCIATED WITH DEGENERATIVE MYELOPATHY OCCURS IN MANY DOG BREEDS

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Keywords: dog, allele frequency, degenerative myelopathy

Background: Canine degenerative myelopathy (DM) is a fatal neurodegenerative disease prevalent in several dog breeds. Typically, the initial progressive upper motor neuron spastic and general proprioceptive ataxia in the pelvic limbs occurs at 8 years of age or older. If euthanasia is delayed, the clinical signs will ascend causing flaccid tetraparesis and other lower motor neuron signs. Previously, a missense mutation has been identified in the canine superoxide dismutase 1 (*SOD1*) gene and showed that homozygosity for the **A**-allele of this SOD1:c.118G > A mutation is a risk factor for DM in five dog breeds: Boxer, Chesapeake Bay retriever, German Shepherd dog, Pembroke Welsh corgi and Rhodesian ridgeback (1). Microscopic examination of spinal cords from affected dogs revealed myelin and axon loss affecting the lateral white matter and neuronal cytoplasmic inclusions that bind anti-SOD1 antibodies.

Objectives: To determine whether this mutation was associated with DM in other breeds and to histopathologically confirm DM in new breeds.

Methods: A TaqMan® SNP allele discrimination assay was used for genotyping. The PCR primer sequences were GTGGGCCTGTTGTGGTATCA and CAAACTGATGGACGTGGAATCC and the probe sequences were VIC-CTCGCCTTTAGTCAGC for the mutant allele and FAM-CGCCTTCAGTCAGC for the wild type allele.

Results: 8,905 individual dogs of 177 distinct breeds or varieties and 110 mixed breed dogs were genotyped at SOD1:c.118 G > A. Genotypes from at least 20 individual dogs were obtained for 120 of the breeds or varieties. In these breeds, the frequencies of the **A**-allele ranged from 90% (Wire fox terrier) to zero (60 different breeds). Nineteen breeds had **A**-allele frequencies greater than or equal to 20%. DM has now been histopathologically confirmed in the following breeds: Pembroke Welsh corgi (A allele frequency = 80%), Boxer (71%), Bernese Mountain dog (45%), Chesapeake Bay Retriever (42%), German Shepherd dog (36%), Kerry blue terrier (35%), Cardigan Welsh corgi (34%), Rhodesian ridgeback (30%), Irish setter (21%) and Standard poodle (5%). Clinical histories resembling DM are common among dogs of certain unconfirmed breeds with high A-allele frequencies: American water spaniel (46%), Canaan dog (31%), Bloodhound (28%), French bulldog (23%) and Kuvasz (21%). Conversely, clinical histories consistent with DM were rare in 6 other breeds with high A-allele frequencies: Wire fox terrier (90%), Pug (39%), Tibetan terrier (31%), Welsh terrier (27%) and Chow chow (21%). All DM affected dogs examined so far have been homozygous for the **A** allele and showed typical histopathology and accumulations of SOD1-immunopositive cytoplasmic inclusions in the spinal motor neurons.

Discussion and Conclusions: The putative existence of dog breeds that have high **A**-allele frequencies but rarely suffer from DM could be explained by genetic modifiers that alter susceptibility to DM.

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THEME 6 EPIDEMIOLOGY

P145 CYANOTOXIN EXPOSURE AS A RISK FACTOR FOR SPORADIC ALS AMONG GULF WAR VETERANS

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Keywords: BMAA, cyanobacteria, cyanotoxins

Background: Epidemiological analysis of ALS cases among personnel deployed to the Gulf between 1990–1991 indicates a significant increase for post-war risk of sporadic ALS with incidence occurring at an earlier age than expected (1, 2). A time-limited outbreak of sporadic ALS occurred in the following decade, which is consistent with latent development of disease after exposure to environmental toxins (3, 4). A meta-analysis of 22 studies indicates increased sporadic ALS incidence in veterans of the Gulf War and of other military deployments (5). This search for a cosmopolitan etiological agent that could account for such exposures during various deployments has focused on β-N-methylamino-L-alanine (BMAA), a motor neuron toxin produced by cyanobacteria (6, 7).

Objectives: To determine if vehicular disturbance of cyanobacterial crusts during military activities within deserts of the Gulf could result in exposure of deployed personnel to BMAA through inhalation.

Methods: Cyanobacterial crusts were collected in the deserts of Qatar. Cyanobacterial taxa were identified and analyzed for BMAA using an Amino Acid Analyzer (AAA), Ultra Performance Liquid Chromatography/Mass Spectroscopy (UPLC/MS) and triple quadrupole Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy (LC/MS/MS). Crusts were artificially aerosolized to simulate vehicular disturbance and the dust analyzed using these methods.

Results: Gulf desert crusts were found to be rich in cyanobacterial genera including Chroococcus, Microcoleus and other species of the Oscillatoriaceae and contained BMAA according to AAA, UPLC/MS and LC/MS/MS analyses. Microcystin was identified through immunological assays and 2,4 diaminobutyric acid (DAB) was also detected in cultured material.

Discussion and Conclusions: Desert dust clouds are effective vectors of toxins from microorganisms (8, 9). Military deployment from 1991-1993 in the Gulf region resulted in significant dust due to vehicular disturbance and other military activities. Exposure of deployed personnel to BMAA, DAB and microcystins may have occurred from inhalation of aerosolized cyanobacterial crusts which may have contributed to the observed increased incidence of sporadic ALS. Cyanobacterial and cyanotoxin exposures through inhalation of dust or ingestion of contaminated water may also account for observed increases of sporadic ALS in other military deployments.

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P146 A SPATIAL ANALYSIS OF ALS IN NEW ENGLAND: RELATIONSHIP TO TOXIC CYANOBACTERIA BLOOMS

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Keywords: cyanobacteria, epidemiology

Background: The incidence of Amyotrophic Lateral Sclerosis (ALS) is approximately two per 100,000 persons. An environmental factor which triggers sporadic ALS is supported by geographic disparities in the incidence of ALS and by the development of the disease in conjugal couples. A high incidence of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS-PDC) has been documented amongst the Chamorro people of Guam and the cyanobacteria toxin beta-methylamino-L-alanine (BMAA), found in components of the Chamorro diet, has been implicated. A high incidence of ALS in Enfield, New Hampshire, which encompasses a lake with a history of documented cyanobacteria blooms, has previously been reported. Exposure to cyanobacteria and cyanotoxins such as BMAA could occur through ingestion of water, consumption of contaminated fish, or inhalation of aerosolized cyanotoxins. Though a number of exposures could be shared by persons in a lakeside community, persons living within close proximity to cyanobacteria blooms may be more likely to be exposed to cyanotoxins and thus may be at a higher risk of developing ALS.

Objectives: To determine if persons living in close proximity to cyanobacteria blooms have a higher incidence of ALS.

Methods: Electronic records and community databases were reviewed to identify the dwelling addresses of patients presenting with ALS between 1998 and April 2009 in New Hampshire (NH), Vermont (VT) and Maine (ME). Case density was adjusted to account for the underlying population density using U.S. Census 2000 data. Records of recent algal blooms were obtained from each individual state's records. ArcGIS 9.3 software was used for spatial analysis. The incidence of ALS over a 10-year interval was calculated within a 0.5 mile (805 meter) buffer zone around

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lakes with documented blooms. This was then compared to the incidence of ALS for persons living farther than 0.5 miles from lakes with cyanobacteria blooms.

Results: Dwelling addresses were obtained for 553 cases of ALS in NH, VT and ME. The odds ratio of developing ALS for persons living within 0.5 miles of a lake with cyanobacterial blooms, compared to the odds for persons living outside this area, was 2.32 in NH (95% CI 1.42–3.80), 1.6 in VT (95% CI 0.9–3.2) and 2.77 in ME (95% CI 1.78–4.31).

Discussion: There appears to be an association between living in close proximity to New England lakes with cyanobacteria blooms and the development of ALS. Limitations of this study include the small number of cases, accuracy of dwelling addresses and the difficulty of retrospectively analyzing environmental factors related to a chronic, progressive disease. It is biologically plausible that exposure of a susceptible individual to a cyanobacterial neurotoxin such as BMAA could result in the development of ALS, however, causation cannot be inferred from a spatial association. Further studies are needed to examine the chronic effects of cyanotoxins.

P147 ESSENTIAL MINERALS AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS ON THE KII PENINSULA, JAPAN

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Keywords: essential minerals, Kii-ALS, risk

Background: Previous epidemiological research in an area of the Kii Peninsula with a high-incidence of ALS (K area) demonstrated low concentrations of Calcium (Ca) and Magnesium (Mg) and high concentrations of toxic minerals, such as Manganese (Mn) or Aluminium (Al) in drinking water and soil compared to those in control areas. Dietary intake of nutrients and serum concentrations of these minerals, however, were not determined in the inhabitants of this area.

Objectives: To examine risk factors for developing ALS in the focus area, with special reference to food intake and essential minerals.

Methods: Blood samples were collected from inhabitants over the age of 40 in the K area (n = 430), the control area (n = 273) and from ALS patients in the Kii Peninsula (n = 95). Serum concentrations of Ca, Mg, Copper (Cu), Zinc (Zn), intact-parathyroid hormone (PTH) and insulin-like growth factor (IGF)-I were determined. A self-administered food frequency questionnaire (FFQ) was used to survey participants who agreed to answer the questionnaire. The differences in mean values or frequencies were statistically examined by unpaired t-test, chi-square test, or Mantel-extension test. The odds ratios (ORs) were estimated using multiple logistic regression models to access the strength of association between ALS and potential risk factors.

Results: A higher proportion of carbohydrate intake and a lower proportion of fat intake was found in ALS patients and in the inhabitants of K area compared to the controls ($P < 0.05$). Dietary intake of Ca was lower in ALS patients than in controls. The ALS patients showed significantly lower concentrations of serum Ca, Cu, Zn and albumin ($P < 0.0001$, respectively) and a tendency toward lower concentrations of intact-PTH compared to those of controls. The inhabitants of K area showed lower serum concentrations of Ca ($P < 0.001$), Cu ($P < 0.02$) and intact-PTH (not significant) and higher concentrations of Zn ($P < 0.05$) than those of controls. There was an inverse relationship between serum concentrations of Ca (lowest vs. highest tertile OR: 0.31 for K area; 0.15 for ALS), Cu (0.92 for K area; 0.12 for ALS) and intact-PTH (0.90 for K area; 0.18 for ALS) and the risk of ALS.

Discussion and Conclusions: It has previously been reported that a high intake of carbohydrate and a low intake of fat increases the risk of ALS. The inhabitants of K area showed similar trends toward dietary intake of nutrients and serum concentrations of essential minerals to ALS patients in the Kii peninsula. These findings suggest that a high proportion of carbohydrate intake with low proportion of fat intake and low serum concentrations of Ca, Cu and intact-PTH might be associated with an increased risk of ALS on the focus area in the Kii Peninsula.

P148 DIETARY PATTERNS AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS IN JAPAN

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Keywords: dietary pattern, meat, epidemiology

Background: Few human studies have reported the relationship between dietary factors and the risk of developing amyotrophic lateral sclerosis (ALS).

Objectives: To examine the relationship between dietary and the risk of developing ALS using a case-control study in Japan.

Methods: The study comprised of 183 ALS patients diagnosed by El Escorial World Federation of Neurology criteria and 407 gender- and age- matched controls that were randomly selected from the general population. A self-administered food frequency questionnaire was used to estimate pre illness intake of food groups and nutrients.

Results: Principal components factor analysis with promax rotation was used to identify 4 patterns that accounted for 38% of the variance of dietary intake frequency: a vegetable-rich pattern, protein-rich pattern (soy products and fish), western-type diet pattern (bread, eggs, red meat and butter) and traditional diet pattern (rice, miso-soup and salted product). Compared to the lowest tertile of the traditional diet pattern, the highest tertile was positively associated with the development of ALS (OR = 2.68; 95% CI: 1.68–4.27; P = 0.001) after adjustment for confounders. The multivariate-adjusted OR for the highest, compared with the lowest tertile of western diet pattern was 0.59 (95% CI: 0.37–0.94; P = 0.02), for the vegetable-rich pattern was 0.64 (95% CI: 0.40–0.95; P = 0.02) and for the protein-rich pattern was 0.48 (95% CI: 0.30–0.75; P = 0.001). Although OR for subjects with only a traditional diet pattern was not significant, that of subjects with traditional diet pattern and one or more dietary patterns among three patterns (vegetable-rich, protein-rich and western-type

diet pattern) were statistically significant (OR: 1.05 for only traditional diet pattern; p for trend = 0.94; OR:5.74 for traditional diet pattern and all three dietary patterns, P = 0.002).

Conclusions: This is the first epidemiological finding that suggests that a dietary pattern with a high intake of carbohydrates such white rice may increase the risk of developing ALS and diets of high-fat and protein foods from animal and vegetable sources may be protective against the onset of ALS.

P149 LIPID PROFILES IN PATIENTS WITH ALS AND ALS MOUSE MODEL

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Keywords: lipid, metabolism, sexual difference

Background: Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of motor and extra-motor systems and its exact mechanism and risk factors are still unclear. Recently, abnormally increased lipid metabolism was found in an animal model of ALS and dyslipidemia was reported to be a prognostic factor in patients with ALS.

Objectives: To investigate the lipid profiles of patients with ALS and mouse models of ALS.

Methods: A review of clinical records and serum lipid profiles and fasting glucose of patients with ALS (n = 95, 60 males, mean age: 54.14 years, mean disease duration: 13.44 months) was performed. Patients with significant dysphagia, duration of disease more than 3 years, or PEG (percutaneous endoscopic gastrostomy) were excluded. Control patients consisted of age-sex matched healthy people (n = 99, 62 males, mean age: 52.52 years). Basal serum lipid profiles and fasting glucose, daily dietary intake and weight were also evaluated in the ALS mouse model.

Results: Basal serum level of cholesterol, triglyceride, LDL/HDL ratio and protein was significantly decreased in patients with ALS (n = 95), compared to controls (n = 99, P < 0.005). This decreased serum level of lipoprotein was only observed in male patients and not in female patients with ALS. As for the ALS mouse model, at early symptomatic stage (90 days of age after birth), nine G93A mutated transgenic mice showed significantly decreased serum level of LDL (11.60 mg/dL in G93A versus 16.44 mg/dL in wild type, P = 0.035) and LDL/HDL ratio (0.20 in G93A versus 0.25 in wild type, P = 0.028), compared to ten control wild type mice. The mean amount of dietary intake (3.95 g/day in G93A versus 3.8 g/day in wild type, P = 0.235) and mean body weight (22.73 g in G93A versus 23.69 g in wild type, P = 0.496) did not differ significantly between these groups.

Conclusions: Patients with ALS and a mouse model of ALS both have a decreased serum level of lipid as compared to controls. However, this is not explained by decreased nutritional intake because the mouse model had intact nutritional intake compared to controls. Further to this, the patients with ALS had a lack of significant dysphagia. Further studies about the central nervous system lipid metabolism in ALS mouse models are in progress.

P150 LEVELS OF CHOLESTEROL AND ALS SURVIVAL: A RETROSPECTIVE SURVEY OF 103 PATIENTS

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Keywords: cholesterol, survival, prognosis

Background: ALS is a degenerative disease of unknown etiology. The presence of high levels of cholesterol and triglycerides has been described in several works, as well as their association with survival.

Objectives: To study the relation between the level of cholesterol and the survival of a group of ALS patients.

Methods: A retrospective survey of 103 deceased patients with an ALS diagnosis, defined according to El Escorial criteria was conducted. The levels of cholesterol were measured through blood analysis, after the onset of the disease and without considering the evolutionary moment. Other factors, which have already been associated with ALS survival, have also been valued: delay of diagnosis, site of onset, age of onset, treatment with Rilutek. Furthermore, other criteria have been introduced to determine the nutritional state at the time of the blood analysis.

Results: Among the 103 patients, 63 of them had a high level of cholesterol. No relation has been found between the level of cholesterol and survival through the statistical analysis. However, the correlation between age, site of onset and delay of diagnosis was significant, as it is already known. In this group of patients, no relation has been observed between treatment with Rilutek and survival.

Discussion: There are discrepancies in the different published works about the correlation between cholesterol and ALS survival. Some authors postulate the presence of metabolic alterations, associating hypercholesterolemia with an increase of survival and questioning the use of statins in those patients. The potential link is unknown.

Conclusions: In the group of patients studied, the statistics do not demonstrate a significant relation between cholesterol and survival. This corroborates the already known relation of factors such as the site and age of onset and the delay of diagnosis. It would be necessary to conduct prospective surveys with serial cholesterol evaluations, nutritional state and lipid metabolism data's evaluations, to confirm or infirm the hypothesis of this relation.

P151 DIFFERENT EFFECT OF STATINS ON THE FUNCTIONAL DECLINE OF MEN AND WOMEN WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: statins, gender influence, functional decline

Background: Treatment with statins has been suggested to possibly increase the incidence of amyotrophic lateral sclerosis (ALS), although a recent analysis by the US Food and Drug Administration (FDA) of data from 41 clinical trials did not show a link between statins and a higher risk of ALS and a

retrospective study did not find a negative effect of statins on survival of ALS patients.

Objectives: To further evaluate whether the use of statins for dyslipidemia has an effect on the rate of functional decline in ALS patients.

Methods: This is a retrospective cohort study based on databases created from files of 594 ALS patients who were assessed for a year. The databases were originally created during two clinical trials by the Northeast ALS consortium which evaluated the efficacy of topiramate and celebrex in ALS patients. The protocols and results of both studies have been published. Since both drugs had no effect on ALS progression compared to placebo, all patients were regarded as placebo treated. For all patients the intake of statins at onset and during the study was known. The functional decline was measured by the ALS Functional Rating Scale (ALSFRS) in the topiramate study and the revised scale (ALSFRS-R) in the celebrex study. The follow-up was performed every two months in the celebrex study and every three months in the topiramate study. The functional decline was compared in each study in patients taking or not taking statins over the follow-up period of one year.

Results: The topiramate trial included 294 patients (189 men, 64.3%), with 26 patients (8.8%) on statins, among them 19 men. The celebrex trial included 300 patients (194 men, 64.7%), 22 (7.3%) of them on statins, among them 15 men. Baseline ALSFRS/-R were similar in statin treated and not treated patients in both trials. As expected, the statin treated patients were older in both studies. The ALSFRS/-R declined significantly with time. There was no significant difference between the functional decline with time of all patients taking statins as compared to that of those not taking statins in each study after adjustment for age. However, the functional decline of women taking statins was significantly greater compared to men taking statins in both studies ($P = 0.005$ in the topiramate trial, $P = 0.028$ in the celebrex trial).

Conclusion: This study indicates that women, in contrast to men, are negatively affected by statin treatment. As women are less affected by ALS and are less represented in clinical trials, analyses that do not take into account gender differences might miss important information.

P152 THE USE OF MORTALITY DATA AS SURROGATE OF INCIDENCE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mortality, incidence, death certificates

Background: Assessing incidence rates for Amyotrophic Lateral Sclerosis (ALS) through morbidity studies is unusual due to uncertain feasibility and high costs. Mortality data have frequently been used to estimate incidence but the accuracy of such a perspective has to be questioned.

Objectives: To identify, through a systematic review, the methodological prerequisite allowing the use of mortality rates of ALS as a surrogate of incidence rates. A Medline literature search was performed to identify (until 2009), studies on ALS and motor neuron disease (MND) mortality rates. The mesh-terms were "ALS", "MND", "mortality", "incidence". Additional references were identified from article citations.

Discussion and Conclusions: Five methodological points have been proposed to be critically assessed when considering a mortality-data-based study. 1) Mortality data must be based on underlying and contributory causes of death. 2) A study (that could be nested in mortality study) about the quality and accuracy of death certificates should be performed or available for the country and period of time that is investigated (true positivity rate, positive predictive value, proportion of unknown causes overall). 3) To compare mortality data among calendar times, using a uniform international classification of diseases (ICD) is essential (ICD 6–8 and 10 on one hand and 9 on the other hand). 4) To compare mortality data among regions, comparable health care systems and death certificate systems (in term of high quality) are essential. 5) To compare mortality data between ethnic groups, critical approach of data (ethnic, access to health care) is indispensable.

P153 THE ACCURACY OF DEATH CERTIFICATION FOR ALS/MND IN IRELAND

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Keywords: death certification, epidemiology, register

Background: Death certification is a recognized means of ascertaining Motor Neurone Disease (MND), although this method has been shown to be less complete when compared to prospective population-based incidence studies.

Objectives: To determine the sensitivity and specificity of death certification for ALS/MND in the Republic of Ireland. The Irish MND Register, which has been in operation for 15 years and seeks to identify all incident patients with MND in the Republic of Ireland using multiple sources of ascertainment, was used to identify all known patients who died from MND from 2002-2006. The Irish Motor Neuron Disease Association was used as a separate verifiable source. The Central Statistics Office (CSO) provided death certificates of individuals over 15 years of age for whom MND (ICD9) was listed as a primary, secondary or tertiary cause of death during the same period.
Cases common or specific to each source were determined to establish the sensitivity and specificity of the death certification process for MND in Ireland.

Results: The cause of death was correctly identified by the CSO as MND in 299 out of 394 (76%) cases known to the Register during the study period. Ninety five (24%) cases known to have died from MND in Ireland were not classified as such on death certification.
Conversely, during the study period, MND was listed as a cause of death by the CSO in 71 cases that were unknown to both the MND Register and the IMNDA database. This represented 20% of all those listed by the CSO. In the majority of these, MND was listed as a secondary/tertiary cause of death and the place of death was more likely to be a rural hospital or nursing home, where diagnostic accuracy is often limited. The absence of demographic detail limited further efforts to verify the diagnosis.

Conclusions: Death certification for MND in Ireland lacks sensitivity and specificity, although the accuracy is similar to that reported in other countries. Prospective identification of

patients using the Irish Register for ALS/MND remains the most accurate method for case ascertainment.

P154 CROSS-SECTIONAL STUDY ON TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

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Keywords: multidisciplinary care, cross-sectional study, treatment

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder. A few treatments are now available including riluzole, respiratory care and nutritional support. Multidisciplinary ALS care can produce important benefits in survival, function and quality of life for patients with ALS. The current situation of the treatment for ALS will be revealed in this study.

Objectives: To find out the treatment situation of ALS in China.

Methods: Data on patients with ALS were collected from 9 ALS or neuromuscular disease centre in different geographical regions in China from 1 February 2009 to 30 April 2009. The treatment history was investigated including treatment with medicines, respiratory care, nutritional support and other multidisciplinary care.

Results: Two hundred and forty patients with ALS were included. They consisted of 152 men and 88 women. One hundred and six patients had clinical definite ALS, 61 with clinical probable ALS and clinical probable ALS-laboratory supported, 44 with clinical possible ALS, 29 with progressive muscular atrophy. At the time of investigation, the mean age was 53.9 ± 12.4 years, ranging from 21 to 88 years; the P_{50} of duration from first symptom to investigation was 12.1 months, ranging from 3 to 84 months. Seventy three patients (30.4%) were treated with Riluzole, P_{50} of the duration of treatment with Riluzole was 2 months (from 0.1 to 21 months); 46 patients (19.2%) stayed on Riluzole since the diagnosis whilst the major reason for Riluzole being discontinued was cost (25.9% of patients). Adverse effects were found in 20% of patients and no effect was found in 31%. Thirty patients (12.5%) received psychological treatment, 10 patients (4.2%) were given antidepressants. Twenty seven patients (11.3%) received physical therapy. Five patients accepted stem cell treatment in two different hospitals. Seventy eight patients (32.5%) would like to accept percutaneous endoscopic gastrostomy (PEG) and 66 patients (27.5%) would like to accept nasal feed. Ninety nine patients would like to accept bimodal positive airway pressure (BIPAP) assistance, of whom 44 patients would like to accept tracheotomy when it is necessary.

Discussion and Conclusions: The treatment with Riluzole in patients with ALS in China is much less than those in developed countries because of economic reasons and no obvious effect. Multidisciplinary care should be emphasized to improve the quality of life for patients with ALS. The percentage of patients who would like to accept tracheotomy and invasive mechanical ventilation is more than those reports from developed counties.

P155 PROSPECTIVE GENETIC AND ENVIRONMENTAL EPIDEMIOLOGICAL STUDY OF ALS

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Keywords: family studies, genetics, environmental epidemiology

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease of unknown etiology. Research suggests ALS is multifactorial in origin, including genetic, environmental and lifestyle factors. Agricultural chemicals, heavy metals, vigorous physical activity, smoking, high glutamate and fat diets have been positively associated with ALS. Prevalence of Parkinson's disease (PD) and Alzheimer's disease (AD) have been reported as higher in ALS patient families than in non-ALS families while other studies report no association. Positive associations of ALS with neurological disease in families may indicate a common genetic or familial origin.

Objectives: To examine genetic, environmental and lifestyle factors associated with ALS and then identify gene-environment interactions.

Methods: ALS patients were enrolled sequentially from an ALS clinic. Patients' siblings, married-in control and married-in control siblings were enrolled. Friend controls enrolled when married-in controls were unavailable. This design provides an opportunity to examine several facets of genetic factors found in ALS patients versus non-ALS subjects. Subjects had blood drawn for cell line or DNA extraction and completed structured interviews on disease history in first degree relatives and environmental exposures. Prevalences of ALS, PD, AD (including related dementias) and Essential Tremor (ET) were measured in all subjects.

Results: From 2005-2009, 101 ALS patients, 72 siblings, 44 married-in controls, 22 siblings of married-in controls and 20 friend controls enrolled. The number of enrolled sibling and control subjects was lower than anticipated. Recruitment of siblings and controls was difficult for several reasons. Either they did not exist, were not contacted by patients, or enrolled but were lost to follow up before data collection. For the family history of neurological disease analysis, information was found on 388 first degree relatives of ALS patients and 266 first degree relatives of controls (married-in and friend controls). Using chi-square methods, no difference was found in the relative risk of ALS, PD and ET relatives of ALS patients versus of other types of unrelated controls. Higher risk of AD was found in friend control relatives versus ALS patient relatives (relative risk $= 0.62$, $P = 0.007$). When data was evaluated on a family level rather than an individual level, there was no significant difference in disease risk between the cases and unmarried controls. Mean ages of the three groups were not significantly different.

Discussion: These results suggest little or no genetic link between ALS and either PD, AD or ET. Type II error is possible given the low number of unmarried controls and low prevalence of these neurological diseases. In addition, the high prevalence of AD in friend controls may indicate some selection bias.

Conclusion: This study design can provide interesting data on genetic and environmental factors associated with ALS. These results also demonstrate the need to consider and minimize control selection bias.

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THEME 7 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P156 FDG-PET STUDY IN AMYOTROPHIC LATERAL SCLEROSIS/PARKINSONISM-DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Keywords: FDG-PET, Kii ALS-PDC

Background: Amyotrophic lateral sclerosis/parkinsonism-dementia complex of the Kii peninsula of Japan (Kii ALS/PDC) is a unique fronto-temporal dementia. The purpose of this study is to reveal glucose metabolism in the brains of Kii ALS/PDC using FDG-PET.

Method: Seven patients with Kii ALS/PDC (one ALS, two ALS with dementia, four PDC) were submitted for the study. FDG-PET was performed on each patient and the data were statistically analyzed by 3D-SSP method.

Result: Glucose metabolism was decreased predominantly in the frontal lobe (frontal gyri, pars opercularis, orbital gyri) and temporal lobe (parahippocampal gyrus, temporal gyri, temporal pole, fusiform gyrus). In some patients, reduced glucose metabolism spread to the parietal lobe (parietal lobules, supramarginal gyrus, angular gyrus, precuneus). The decrease of glucose metabolism in the cingulated gyrus was seen in all patients.

Conclusions: In Kii ALS/PDC, the reduction of glucose metabolism was detected mainly in the frontal and temporal lobes, extending to the parietal lobe. The decrease of glucose metabolism in the cingulated gyrus was an essential finding in Kii ALS/PDC.

P157 EXECUTIVE FUNCTION AND FRONTOTEMPORAL DEGENERATION IN ALS AND ALS-FTD

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Keywords: FTD, imaging

Background: A sizable percentage of ALS patients possess a fronto-temporal syndrome. We therefore hypothesize that this reduced cognitive function reflects fronto-temporal degeneration across the spectrum of ALS and ALS-FTD.

Objectives: To correlate neuroimaging with neuropsychological testing in patients with ALS and ALS-FTD.

Methods: Twenty-one patients (10 ALS, 9 ALS-FTD, and 2 ALS with executive dysfunction) were studied with neuropsychological tests. Diagnoses of FTLD were made in a multidisciplinary team conference using Neary Criteria. MRI exams were performed on a 1.5T Scanner. Templates of lobar grey matter and white matter (determined from DTI connectivity) were registered to the patients' T1-weighted volumes ($1 \times 1 \times 1.5$ mm^3) and DTI data ($2.2 \times 2.2 \times 2.2$ mm^3) to determine grey matter volume (GV), white matter mean diffusivity (MD) and fractional anisotropy (FA), respectively. Anova ($p < 0.05$) were used for cross-sectional comparisons of MRI metrics between ALS and ALS-FTD cohorts after removing age and gender effects. Stepwise regression models of cognitive variables as functions of lobar GV, FA and MD with age and gender as covariates were performed.

Results: Frontal grey matter volume was decreased and right temporal white matter MD increased in ALS-FTD compared to ALS. For the MMSE and CVLT-SF there were no significant correlations. For verbal-fluency the following correlations were obtained: R Frontal-GV (RSQ: 0.31; $p < 0.05$), L Frontal-FA (RSQ: 0.41; $p < 0.008$), R Temporal-GV+FA (RSQ: 0.46; $p < 0.008$), and L Temporal-GV+FA (RSQ: 0.45; $p < 0.008$). For DKEFS-Trails the following correlations were obtained: R Frontal MD (RSQ: 0.52; $p < 0.05$), L Frontal-MD (RSQ: 0.45; $p < 0.008$), R Temporal-GV (RSQ: 0.67; $p < 0.001$), L Temporal-MD (RSQ: 0.36; $p < 0.008$). Boston naming and DKEFS-Stroop both correlated significantly with MD for the right frontal lobe and with GV for the left frontal and bilateral temporal lobes ($p < 0.008$).

Discussion and Conclusions: Phonemic verbal fluency correlated with grey and white matter abnormalities, as did a verbal inhibition task (Stroop) and a test of mental flexibility (Trails). These results demonstrate that impaired cognition is associated with increased fronto-temporal grey and white matter degeneration in ALS-FTD compared to ALS patients.

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P158 HIPPOCAMPAL ATROPHY RELATED TO MEMORY PERFORMANCE IN NON-DEMENTED ALS PATIENTS: A VOXEL BASED MORPHOMETRY AND NEUROPSYCHOLOGICAL STUDY

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Keywords: hippocampus, memory, VBM

Background: In patients with amyotrophic lateral sclerosis and frontotemporal lobar degeneration (ALS-FTLD), neuropathological studies have shown abnormalities in the medial temporal lobe. In 20–30% of ALS patients (without FTLD) cognitive dysfunction, including memory impairments, have been found. The nature of structural brain abnormalities, if

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present, underlying memory dysfunction in non-demented ALS patients *in vivo* is unknown.

Objectives: To investigate global and regional brain atrophy in relation to memory function in non-demented ALS patients, using voxel based morphometry (VBM) of MRI images.

Methods: T1-weighted 3T MRI images were obtained from 27 non-demented ALS patients (6 bulbar onset, 21 limb onset) and 23 age, education and gender matched healthy controls. Global and regional brain atrophy was quantified using voxel based morphometry, implemented in SPM5. After automated segmentation, the Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra was used for registration and normalisation of the grey matter images. Patients and controls underwent a neuropsychological assessment adapted to motor impairment, including tests of memory, executive functions, language and attention. Patients had a vital capacity above 70% of the predicted value. Normative data were used to calculate frequencies of cognitive dysfunction (defined as performance lower than 2 SD below the mean, corrected for age and level of education). Global brain atrophy was quantified and the presence of regional brain atrophy was investigated in relation to cognitive measures.

Results: Neuropsychological investigation: Comparison with normative data revealed that both Immediate and Delayed Recall subtests of the Logical Memory subtest of the Rivermead Behavioural Memory Test (RBMT) were most frequently (19%) abnormal in ALS patients ($n=26$), compared to other cognitive tests. As a group, ALS patients performed worse on an attention/working memory test (letter-number sequencing) and naming. VBM: Overall, ALS-patients showed reduced volume of the right dorsolateral prefrontal cortex and the superior frontal gyrus compared to controls. In ALS patients, performance on the RBMT was correlated to bilateral hippocampal volume (L: $Z=4.40$, $p<0.001$, uncorrected; R: $Z=3.72$, $p<0.001$, uncorrected) and left insula volume ($Z=4.06$, $p<0.001$). These correlations were not found in controls. No differences were found in white matter volumes, or in grey matter volume of the precentral gyrus.

Conclusions: Non-demented ALS patients with mild cognitive dysfunction show bilateral grey matter hippocampal atrophy related to verbal memory function. In addition, two regions in the prefrontal cortex, reported earlier to be involved in non demented ALS patients, show grey matter atrophy. These results provide further support for extramotor involvement, including the medial temporal lobes, in non-demented ALS patients.

P159 7 TESLA MRI SHOWS NO SIGNS OF BLOOD-BRAIN BARRIER DYSFUNCTION IN ALS

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Keywords: 7 Tesla MRI, blood-brain barrier dysfunction, microbleeds

Background: Recently there have been several reports about disruption of the blood-spinal cord barrier (BSCB) in SOD1 transgenic mice (1–3). A reduction of tight junction proteins results in microhemorrhages and the release of neurotoxic haemoglobin-derived products as well as reductions in microcirculation and hypoperfusion. In a later phase inflammatory changes occur (1). We investigated blood brain barrier (BBB) disruptions in early stage ALS with susceptibility weighed imaging (SWI) on 7 Tesla MRI.

Objectives: To investigate the integrity of the BBB in ALS by quantification of microbleeds or hemosiderin depositions.

Methods: Twelve patients with ALS (probable lab-supported, probable or definite ALS according to the El Escorial criteria) and 12 age and sex matched healthy controls were studied. The patients were recently diagnosed with ALS and subjects with vascular comorbidity were excluded. Clinical status was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R). We performed SWI on a Philips 7 Tesla MRI scanner. With this type of imaging hemosiderine deposits of less than 0.5mm can be detected. Two blinded physicians counted the microbleeds.

Results: Sporadic microbleeds were detected in the patient group and the control subjects. No differences were found between the two groups.

Discussion and Conclusions: There is no evidence for BBB disruption in early stage ALS based on SWI with high field MRI. Further research is needed to reveal the role of BBB disruptions in the pathogenesis of ALS.

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P160 CHANGES IN CORTICAL AND SUBCORTICAL MOTOR ACTIVITY IN AMYOTROPHIC LATERAL SCLEROSIS: A FMRI STUDY AT EARLY DISEASE STAGE AND DURING ITS PROGRESSION

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Keywords: fMRI, motor activity

Background: A suitable method to study the dynamics of neurodegeneration and possible compensatory processes in ALS is functional imaging. The neurodegenerative process is already advanced before muscle weakness and wasting occurs. In this study we investigated the motor activations using fMRI with a focus on unaffected limbs. We also compared these data with those from more progressed disease status.

Methods: Two groups were investigated using BOLD-fMRI, while they performed a blocked motor task (finger flexion and extension of fingers in the right hand against rest). Imaging was performed in a 3T system. fMRI data were analyzed with Brain Voyager QX. The first group comprised of 22 healthy volunteers (12 women) aged from 42 to 67 years (mean age 61 years). The second group consisted of 22 patients (9 women) who fulfilled the diagnostic criteria for probable or definite ALS according to the revised El Escorial criteria of the World Federation of Neurology. The mean ALSFRS-R score was 39.5 (range 34 to 45). The interval between the diagnosis and

the study was 6 months (range 1 to 15). Medical Research Council (MRC) scale was used for evaluation of muscle strength. Grip strength of the right hand was evaluated using Martin-Type Squeeze Dynamometer (Vigorimeter, Martin, Tübingen, Germany).

Results: During finger movement we found significant activations in primary motor and premotor cortex (precentral gyrus; BA 4, 6); somatosensory cortex (postcentral gyrus; BA 2, 3); supplementary motor area (SMA, BA 6) and subcortical areas in all groups. The contralateral cortical and subcortical activity in ALS patients was significantly increased compared to the control group even for clinically unaffected limbs. Cluster volumes of contralateral activity were highly similar for ALS patients in different stages of disease and showed no correlation to muscle atrophy or weakness in the performing right hand. However the Beta weight of these clusters had a negative correlation with disease progression and a positive correlation with ALSFRS-score. The rate of ipsilateral sensorimotor activation was increased in ALS and the volume was negatively correlated to weakness in the right hand.

Conclusions: The increased cortical activity in ALS patients for movements with clinically unaffected limbs demonstrates adaptive changes in cortical activity before the manifestation of clinical signs. Decrease of Beta weight in the contralateral sensorimotor area and increase of cluster volume of the ipsilateral sensorimotor area are additional functional changes during disease progression.

P161 CORTICAL FINGERPRINT AND MOTOR NETWORK DEGENERATION IN ALS

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Keywords: diffusion tensor imaging, cortical thickness, dying forward

Background: Motor neuron and corticospinal tract (CST) degeneration are key pathological features of amyotrophic lateral sclerosis (ALS). Automated whole brain cortical thickness measurements can provide objective information on the pattern of neurodegeneration and the involvement of motor areas. The specific pattern of neurodegeneration could be a surrogate marker for ALS (1). We evaluated the involvement of the motor network by measuring the cortical thickness in the precentral gyrus together with diffusion tensor imaging (DTI) on the CST and the corpus callosum.

Objectives: To explore the pattern of neurodegeneration in ALS. To estimate the degree of motor network degeneration based on cortical thinning in the motor areas and DTI of the CST and the corpus callosum.

Methods: Twelve patients with ALS (probable lab-supported, probable or definite ALS according to the El Escorial criteria) and age and sex matched healthy controls were studied. Clinical status was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R). Cortical thickness and DTI data was acquired on a 3 Tesla Philips Achieva Medical Scanner. We used a validated and automated method to measure whole brain cortical thickness. We measured fractional anisotropy (FA) values along the CST and the corpus callosum, using tract-basted statistics (2).

Results: We found two regions of significant cortical thinning in the primary motor areas in ALS. These regions correspond to the arm and leg region respectively. Slightly reduced FA values were found along the corpus callosum and the CST in ALS compared to controls. Interestingly, exploratory analysis suggest that this reduction gradually decreases as the CST descends from the cortex to the brainstem. We found the average cortical thickness in the precentral gyrus to be associated with the FA values in the rostral part, but not in the caudal part of the CST. There was also an association between cortical thickness and FA values in the corpus callosum.

Discussion and Conclusions: This study gives an impression of the pattern of neurodegeneration and an indication for motor network degeneration in ALS. This degeneration seems to occur in an anterograde manner. This finding gives new insights into the mechanism of neurodegeneration. The advanced analysis technique permits detailed quantification of FA values along a neural tract. The association of FA values with cortical thickness supports the proposed mechanism.

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P162 CHANGES OF FUNCTIONAL CONNECTIVITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: fMRI, resting-state

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving primarily the upper and lower motoneurons with a rapid progression. Even early descriptions of ALS pointed out that some patients develop dementia and, more recently, neuropsychological, electrophysiological, and neuroimaging results suggest that the disease process involves other parts of the nervous system. The demonstration of functional involvement of brain networks outside of the motor system proper is of great importance for our understanding of ALS. The present contribution seeks to provide evidence for such extra-motor involvement using independent component analysis (ICA), applied to blood oxygen level-dependent (BOLD) time-series obtained during rest.

Methods: A functional imaging approach was used, involving the analysis of resting state activity, followed by the definition of functionally connected brain networks by ICA to assess differences between ALS patients (n = 20) and healthy controls (n = 20). Analysis and visualization of the data were performed using Brain Voyager QX (Brain Innovation BV, Maastricht, The Netherlands) software.

Results: ICA analysis revealed 5 typical brain networks among which two, the so-called default mode network and the sensori-motor network showed distinct differences between patients and controls. The default mode network showed less activation in patients in several regions including the ventral anterior cingulate cortex, posterior cingulate cortex and the left and right inferior

parietal cortex, regions that have been linked previously to executive functions. The sensori-motor network showed group differences in the premotor cortex. As in the present approach no task is imposed on the subject, these data favour a primary functional involvement of the premotor cortex in ALS.

Conclusions: Analysis of resting state network activity in ALS allowed the demonstration of significant changes in two out of five studied networks, the default mode and sensori-motor networks. The former has been linked to cognitive processes, whereas the latter has been demonstrated to be involved in motor control. The present results once again demonstrate extra-motor involvement in ALS. Among the decisive advantages of the resting state approach is the fact that no task is imposed on the subjects and thus no compensatory processes (e.g. increased effort) have to be considered. Further advantages include the brief examination time and the ability to investigate several networks at the same time.

P163 DIFFUSION TENSOR IMAGING OF THE CORTICOSPINAL TRACT IN RECENT ONSET MOTOR NEURON DISEASE: A LONGITUDINAL STUDY

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Keywords: MRI, diffusion tensor imaging, subtypes

Background: Amyotrophic lateral sclerosis (ALS) is a disease of upper (UMN) and lower (LMN) motor neurons. Whether the initial target of disease is the UMN, LMN, or both simultaneously, has not been settled. ALS and other motor neuron diseases such as primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA) may have different etiologies or different disease modifying factors. Therefore, several scenarios of disease onset and progression may be studied in these phenotypes. Diffusion tensor imaging (DTI) is a surrogate marker for UMN degeneration. Decreased anisotropy suggests diminished integrity of a tract and thus is a surrogate marker for axonal loss. Diffusion tensor tractography allows for the *in vivo* investigation of white matter tracts.

Objectives: To focus on UMN involvement as measured with diffusion tensor tractography of the corticospinal tract (CST) in recent onset ALS and PMA.

Methods: In a prospective cohort study we included patients with bulbar onset ALS (ALS-B), limb onset ALS (ALS-L), PMA, PLS and healthy controls, 12 individuals per group. Patients with ALS and PMA had weakness for less than one year. All patients were seen at baseline and 6 months later, except for the PLS patients who were only seen at baseline. Main clinical outcome measures were the revised ALS functional rating scale (ALS-FRS-R) and finger tapping speed. Diffusion tensor MRI data were acquired by means of a spin-echo EPI sequence. We computed fractional anisotropy (FA) values from the eigenvalues. We reconstructed the corticospinal tract bilaterally in DTI-Studio. FA profiles were derived along the caudo-cranial course of the CST. Volumetric changes in grey and white matter were studied by means of Voxel Based Morphometry (VBM) of three dimensional T1- weighted MR images using DARTEL. Statistical significance for all analyses was set at $p < 0.01$).

Results: Disease rapidity did not differ significantly in PMA from that in ALS-L and ALS-B. In PLS we found a significantly decreased FA along the entire course of the CST. In ALS-L we found a significantly decreased FA only in the centrum semiovale at baseline, whereas at follow-up there was a trend towards a further decrease more caudally. In ALS-B FA was significantly decreased in the caudal CST (pons/mesencephalon) and in the posterior limb of the internal capsule. At follow-up there was a trend towards a further decrease more cranially. In PMA FA showed neither a decrease along the CST compared to controls nor a decrease over time. In PLS only we demonstrated volumetric changes in PLS in the subcortical white matter of the primary motor cortex. Finger tapping speed correlated strongly with FA values. FA change over time was not correlated with a change in any of the clinical variables.

Discussion: We demonstrated CST degeneration at baseline in PLS and both ALS groups but not in PMA, with a trend towards further degeneration at follow-up only in both ALS groups. FA values correlated strongly with finger tapping speed.

P164 REGIONAL WHITE MATTER ALTERATIONS IN RARE MOTOR NEURON DISEASES: A WHOLE BRAIN-BASED ANALYSIS BY USE OF DIFFUSION TENSOR IMAGING METHODS

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Keywords: diffusion tensor imaging, Primary Lateral Sclerosis, Hereditary Spastic Paraparesis

Background: Different motor neuron disorders (MNDs) are mainly defined by the clinical presentation based on the predominance of upper or lower motor neuron involvement and the course of the disease. To date, magnetic resonance imaging (MRI) has mostly served as a tool to exclude other pathologies, but step by step novel approaches based on volumetric/morphometric techniques, magnetic resonance spectroscopy and, most recently, diffusion tensor imaging (DTI) have started to add information on the underlying pathophysiological processes of these disorders *in vivo.*

Methods: The present study was designed to investigate three different MND, i.e. primary lateral sclerosis (PLS, N = 25), pure hereditary spastic paraparesis (HSP, N = 24), and X-linked spinobulbar muscular atrophy (X-SBMA, N = 20), by application of whole-brain based DTI analysis methods in comparison with matched controls. All data analyses were performed by use of the DTI software TIFT (Tensor Imaging and Fiber Tracking).

Results: The analysis of white matter (WM) involvement revealed widespread and characteristic patterns of alterations within the motor system with varying predominance

according to the clinical focus. There were also WM changes in projection to the limbic system and within the corpus callosum, the latter both for HSP and, unexpectedly, for PLS. In detail, multiple areas of significantly reduced fractional anisotropy within the supratentorial motor system were delineated in the PLS group and in the HSP group, and to a lesser degree in patients with X-SBMA. In addition, the X-SBMA group showed large involvement of the limbic projectional system which was less widespread in HSP and PLS.

Discussion: In summary, DTI was able to delineate a characteristic WM pathoanatomy in motor and extra-motor brain areas for different MND via whole brain-based fractional anisotropy assessment. Future advanced MRI-based investigations might help to provide a fingerprint-identification of MND.

P165 AN ALGORITHM TO DETERMINE UPPER MOTOR NEURON INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: corticospinal tract, transcranial magnetic stimulation, diffusion magnetic resonance imaging

Background: The diagnosis of Amyotrophic Lateral Sclerosis (ALS) relies on concomitant upper and lower motor neuron dysfunction in one or more body regions. In some patients, upper motor neuron (UMN) features may be subclinical, potentially resulting in misdiagnosis.

Objectives: The present study combined novel threshold tracking transcranial magnetic stimulation (TMS) with diffusion tensor imaging (DTI) techniques to develop an objective marker of UMN dysfunction.

Methods: Studies were undertaken in 10 ALS patients and results were compared to 10 normal controls. TMS studies were performed using a 90 mm circular coil connected to a BiStim device, with magnetic evoked potentials recorded from abductor pollicis brevis. Fifteen directional DTI scans were obtained using an isotropic voxel resolution of 2.5 mm.

Results: Short interval intracortical inhibition (SICI) was significantly reduced in ALS patients (averaged SICI 1–7 ms ALS $-2.1 \pm 0.8\%$; controls $7.1 \pm 1.2\%$, $P < 0.0001$). This reduction in SICI was associated with reductions in intracortical facilitation and cortical silent period duration, thereby re-affirming the presence of UMN dysfunction in ALS. DTI demonstrated significant reduction in fractional anisotropy (FA) along the corticospinal tract (CST) (left CST ALS 0.58, control 0.62, $P < 0.01$ and right CST ALS 0.57, control 0.60, $P < 0.01$). There was also an increase in trace apparent diffusion coefficient (trace ADC) and perpendicular diffusivity (Perp D), consistent with fibre tract degeneration. There was significant correlation between SICI and DTI parameters, suggesting that SICI was associated with fibre tract degeneration. Based on these correlation studies, an UMN index was developed, which was more sensitive than conventional DTI findings at detecting UMN abnormalities long the CST.

Discussion and Conclusions: TMS and DTI studies have established simultaneous functional and structural abnormalities of the UMN system in ALS. The UMN index may be refined as a diagnostic and prognostic marker in ALS.

P166 EVIDENCE FOR WIDESPREAD GREY AND WHITE MATTER VOLUME DECREASES IN PRIMARY LATERAL SCLEROSIS: A VOXEL-BASED MORPHOMETRY STUDY

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Keywords: voxel-based morphometry, Primary Lateral Sclerosis, GM/WM alterations

Background and Objectives: Motor neuron disorders reflect a heterogeneous group of neurodegenerative diseases, which tend to follow a more or less slow course. Due to differences in prognosis and therapeutic aspects, biomarkers are needed to discriminate different disease entities. In primary lateral sclerosis (PLS), clinical diagnostic criteria were defined and supportive laboratory tests help to exclude other diseases. Magnetic resonance imaging (MRI) and further advanced imaging techniques (e.g. voxel-based morphometry, VBM) are of increasing interest due to their potential to elucidate neuropathological alterations *in vivo*.

Methods: We evaluated 25 patients with PLS (57.0 ± 12.5 years), recruited from our outpatient clinic for MND at the Department of Neurology of the University of Ulm, Germany. All patients underwent standardized clinical, neurological and routine laboratory examinations and met the diagnostic criteria for PLS. 3-D T1-weighted data sets (MP-RAGE) were acquired on a 1.5 Tesla clinical MRI scanner and complete postprocessing for grey and white matter separately was performed according to the optimized VBM protocol by use of SPM2 (Statistical Parametric Mapping).

Results: Voxel clusters of significantly decreased grey matter volumes ($p < 0.05$, corrected) were localized in the superior frontal gyrus in close proximity to the parasagittal cortical representation area of the lower limbs, within the nucleus ventralis posterolateralis of the right thalamus and in projection to the middle frontal gyrus of the right hemisphere, respectively. The WM analysis revealed widespread clusters of significantly decreased WM volume in subcortical brain areas adjacent to the motor system at $p < 0.05$, corrected, with a slight preponderance of the right hemisphere.

Discussion: Neuroimaging evidence was obtained for structural GM and WM alterations in patients with PLS. These results are in accordance with the proposition that the most prominent pathologic hallmark in PLS is reflected by the loss of pyramidal neurons in motor and premotor cortical areas and associated axonal degeneration. Particularly with regard to the widespread WM alterations adjacent to the cortical representation areas of the motor system of the lower limbs, these results may serve as a fingerprint-characterization for PLS in comparison to other MND.

P167 UTILIZING DIFFUSION TENSOR IMAGING (DTI) FOR THE ASSESSMENT OF UPPER MOTOR NEURON INVOLVEMENT IN FLAIL LEG VARIANT ALS

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Keywords: flail leg syndrome, diffusion tensor imaging, UMN involvement

Background: The flail leg ALS variant is a lower motor neuron syndrome characterized by progressive distal onset weakness and wasting restricted to the lower limbs for at least 12 months. It can be identified in 3–6% of all ALS cases. Patients survive, on average, twice as long in comparison to classical ALS patients. The integrity of upper motor neuron projections in the flail leg variant of ALS has yet to be studied. Fractional anisotropy (FA) measures, derived from diffusion tensor weighted imaging (DTI), serve as sensitive *in vivo* markers of the integrity of cerebral fibre tracts.

Objectives: To assess the degree of degeneration of the intracranial portion of the corticospinal tract in flail leg variant ALS in comparison to classical ALS using DTI.

Methods: We studied two patients with flail leg syndrome (aged 55 and 60 years; ALSFRS-R 36-, 38/48, disease duration 40 and 26 months, respectively). For comparison, we studied 5 patients with the clinical diagnosis of definitive ALS (aged 47–61 years; ALSFRS-R 12–42/48, median 27/48; disease duration 10–54 month, median 14), using DTI at 1.5 Tesla. After selection of a seed region of interest, we tracked the nerve fibres along the dorsal pyramidal tract which represents the projection site for the legs. FA along these fibre tracts was averaged and individually compared between the flail leg patients and the classical ALS controls using z-score transformation.

Results: Among the 2 patients with flail leg ALS relative to the classical ALS controls, the z-scores of FA values were (right/left) 0.9 and 1.6 for the one, and 1.5 and 3.3 for the other patient. This suggests that the fibre tracts are relatively preserved in the dorsal part of the pyramidal tract.

Conclusions: The fibre tracts representing projections from the upper motor neuron of the legs were relatively preserved in patients with flail leg syndrome relative to classical ALS patients. These findings support the notion that DTI is a significant tool to measure the integrity of cerebral fibre tracts in neurodegenerative disorders.

P168 ELECTRICAL IMPEDANCE MYOGRAPHY AS A PRE-SYMPTOMATIC BIOMARKER OF ALS

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Keywords: electrical impedance myography, biomarker, pre-symptomatic

Background: Amyotrophic lateral sclerosis is characterized by a pre-symptomatic phase during which neuronal degeneration occurs prior to the appearance of clinical symptoms. The unknown duration of this preclinical phase as well as the possibility that motor neuron loss is extensive prior to the appearance of symptoms represent serious challenges to a better understanding of the cause and risk factors for ALS, and to the development of effective therapies. These observations provide a rationale for the identification of biomarkers of the preclinical phase of the disease that might be used to permit early diagnosis and initiation of therapy earlier in the course of the disease.

Objectives: To evaluate the utility of electrical impedance myography (EIM), a novel electrophysiological technique that is sensitive to changes in the biophysical properties of muscle, as a biomarker of pre-symptomatic disease.

Methods: The Pre-Familial ALS (Pre-fALS) study is an ongoing prospective observational study of people at risk for developing ALS. Asymptomatic individuals from SOD1 positive (SOD+) fALS pedigrees are recruited and evaluated annually. Both SOD1+ and SOD1− family members are included. Multi-frequency EIM was performed by an evaluator blinded to SOD1 mutation status. EIM involves the application to a limb of a low-intensity, alternating current at a spectrum of frequencies ranging from 10 kHz to 1 MHz with measurement of the resulting voltages across a pair of electrodes placed over a muscle of interest. EIM provides both a measure of the offset in timing of current flow produced by the cell membranes (the reactance) and a measure of the obstruction to current flow offered by intracellular and extracellular fluid (the resistance). EIM data for the spectrum of frequencies were collapsed into single values by calculating the slope of the reactance values across a range of frequencies. Subjects were categorized as likely SOD1+ or likely SOD1− based on EIM results by an independent blinded evaluator.

Results: Twenty-five participants were studied, including 17 SOD1+ and 8 SOD1− subjects. Mean age (range) in the SOD1+ group was 48 (27–63) compared to 49 (31–64) in the SOD1− group. There were more females in the SOD1+ group (76% vs 50%). Height and weight were comparable between the two groups. Fourteen of the 17 SOD1+ subjects were correctly classified as such based on the results of EIM alone (sensitivity 82%). Five of the eight SOD1− subjects were correctly classified as such based on EIM alone (specificity 62.5%). The kappa statistic is 0.44, supporting moderate agreement between the two tests.

Discussion and Conclusions: These cross-sectional data suggest that the EIM reactance slope may be a useful biomarker of pre-symptomatic disease in SOD1+ individuals. Confirmation and validation of these findings will await the results of our ongoing longitudinal evaluation of the Pre-fALS study cohort.

P169 ASSESSING ALS PROGRESSION OVER SHORT TIME PERIODS WITH FREQUENT MEASUREMENTS: A COMPARISON OF ELECTRICAL IMPEDANCE MYOGRAPHY, HANDHELD DYNAMOMETRY AND ALSFRS-R

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Keywords: biomarker, electrical impedance myography, handheld dynamometry

Background: Electrical impedance myography (EIM) is a technique for the assessment of neuromuscular disease that relies upon the application of high-frequency, low-intensity electrical current to localized areas of muscle and the measurement of the resulting surface voltages. To date, EIM

has shown good reproducibility and a high sensitivity to progressive neurogenic change in muscle. For this reason, it was reasoned that EIM may be capable of detecting muscle deterioration in ALS over short periods of time. Ultimately, since the technique is easy to apply, it could serve as a useful outcome measure in ALS clinical trials.

Objectives: To assess EIM's potential to detect ALS disease progression over short time periods in comparison to hand-held dynamometry (HHD) and the ALSFRS-R.

Methods: Two ALS patients were recruited as part of a multi-center study evaluating EIM in ALS. The first was a 64-year-old man with sporadic disease and slow progression and the second was a 44-year-old woman with the SOD1 A4V mutation and clinically more rapid progression. EIM, handheld dynamometry, and ALSFRS-R data were obtained as frequently as every 2 weeks for a period of several months. EIM measurements were made over several upper and lower extremity muscles on one side. HHD was performed bilaterally.

Results: In the slowly progressing patient, at the end of 3 months, EIM phase remained stable, but for the most rapidly progressing muscle, a reduction of 18% was observed. ALSFRS-R had increased by 2.5% and mean HHD showed a 5% reduction. For the more rapidly progressing patient, at the end of 6 weeks, mean EIM phase declined 17.1%, but for the most rapidly progressing muscle, a 42% decline was observed. In comparison, mean HHD demonstrated a 21.2% decline with an 8% decline in ALSFRS-R. In the patient with slow progression, EIM phase appeared to drift up and down over a several week period at different times in several muscles.

Discussion: These findings support that reductions in EIM as well as in HHD can be detected over relatively short periods of time in even slowly progressing patients. Moreover, frequent monitoring of ALS patients may reveal previously unappreciated variations in outcome measures over a period of just several weeks. Specifically, the fluctuations observed in the EIM data in the slowly progressing patient may represent evidence of ongoing denervation and subsequent reinnervation.

Conclusions: Intensive monitoring of ALS with EIM and HHD may provide novel insights into disease progression. These techniques could be used either independently or together to help assess disease progression over just 2–3 month time periods, offering an approach to shortening the length of Phase II clinical trials whilst also providing a new tool to study disease biology.

P170 DOES LMN LOSS IN ALS DERIVE FROM PRIMARY UMN DEGENERATION? THE STERNOCLEIDOMASTOID-TRAPEZIUS MUSCLE MODEL

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Keywords: upper motor neuron, lower motor neuron, trans-synaptic degeneration

Background: The concept that ALS commences in the UMN, and that LMN loss results from a trans-synaptic degenerative process, has been supported by the finding of early cortical hyper-excitability, although there is no classical pathological support for this suggestion. One approach to this concept is to investigate ipsilateral trapezius (TM) and sternocleidomastoid (SCM) muscles in the same patients. Since the TM receives UMN input from the contralateral motor cortex and SCM from both hemispheres, as shown by transcranial magnetic stimulation, asymmetry of LMN changes in these two muscle would support the concept of primary UMN degeneration.

Objectives: To study the role of UMN in LMN degeneration.

Methods: Forty-two normal control subjects and 62 age and sex-matched ALS patients with definite or probable disease (mean age 62 years, SD 14: mean disease duration 12.9 months, SD 7.2; 24 bulbar-onset) were investigated. The diagnosis of ALS was established at the time of the first EMG evaluation and clinical follow-up confirmed the diagnosis. MUP analysis (mean amplitude, duration and% of polyphasic potentials) was studied in TM and SCM in the more severely affected upper limb in each patient (right side when both were equally affected). In 11 patients in whom clear asymmetrical upper limb involvement was observed both sides were fully investigated to test symmetry. Non-parametric tests were applied and $p < 0.01$ was considered significant.

Results: In controls, SCM mean amplitude (0.53 mV vs 0.61 mV) and duration (9..88 ms vs 10.7) were significantly lower than in TM, but % polyphasic potentials was greater in SCM (16.61 vs 9.88). No spontaneous activity was observed in controls. In ALS patients amplitude, duration and % polyphasic potentials were significantly greater as compared with controls for both muscles. In SCM 6.5% and 55% showed fibs-sw and fasciculation potentials (FPs) respectively. In TM in these patients these values were 14.5% and 69% respectively, a non-significant difference. Regarding MUP analysis mean amplitude (0.91 mV vs 1.08) and duration (11.87 ms vs 12.72) were higher for TM and % polyphasic potentials (25.9 vs 15.5) higher for SCM, compared to controls; in addition the percent differences in MUP measurements between these muscles were similar in controls and patients with ALS. In the group of 11 patients in whom both sides were investigated there was a non-significant asymmetry in the tested measurements.

Discussion: TM and SCM are innervated by the same nerve and are anatomically adjacent. Both are recruited in voluntary movement and posture, and also as accessory respiratory muscles. However, their corticospinal input is different. These results do not support a primary role for UMN pathophysiology at the onset of ALS, although we have confirmed that these muscles are affected early in ALS and show frequent FPs, which are useful to confirm diagnosis.

P171 THE NEUROPHYSIOLOGICAL INDEX – PERFORMANCE IN PHASE II CLINICAL TRIALS

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Keywords: neurophysiological index, clinical trial, biomarker

Background: The neurophysiological index (NI) was devised as a measure of peripheral disease burden in amyotrophic lateral sclerosis (ALS). It undergoes more rapid decline than other clinical measures of disease progression, including

forced vital capacity and functional rating scales. As such, the NI has been proposed as an electrophysiological endpoint for clinical trials in ALS.

Objectives: To evaluate the performance of the NI in two Phase II clinical trials in ALS.

Methods: Standard nerve conduction studies were undertaken on the ulnar nerve-abductor digiti minimi system at the wrist in 34 patients with ALS (22 males and 12 females, aged 54 ± 1.5 years). Testing was undertaken on four occasions over 12 weeks. The NI was calculated according to the formula: compound muscle action potential (CMAP; mV) F-wave frequency (%) / distal motor latency (DML; ms). Patients were also scored with the Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALS FRS-r). Normative electrophysiological data was also collected from 22 healthy control subjects (5 males and 17 females; aged 41.8 ± 14.5 years).

Results: Assessment of the NI was brief (completed within three minutes) and was successfully undertaken in all ALS patients, confirming feasibility in an ALS cohort. The CMAP (7.5 ± 0.4 mV), DML (2.8 ± 0.1 ms), F-wave frequency (81 ± 4%) and NI (2.4 ± 0.2) were reduced at baseline, in comparison to healthy control subjects (CMAP, 11.5 ± 0.6, $P < 0.00001$; median F-wave frequency, 100%, $P < 0.00001$; DML, 2.3 ± 0.1 ms, $P = 0.0001$; NI, 4.9 ± 0.3, $P < 0.001$). After 12 weeks follow-up, CMAP (7.0 ± 0.44; $P = 0.002$), F-wave frequency (0.7 ± 0.05; $P = 0.002$) and NI (1.89 ± 0.22; $P < 0.0001$) were significantly reduced. By contrast, DML increased significantly (2.9 ± 0.07; $P = 0.03$) at the end of the 12-week observation period. Over this period, the ALS FRS-r underwent a marginal reduction of 5% (baseline, 40.6 ± 0.7; week 12, 38.6 ± 0.83; $P < 0.00001$).

Discussion and Conclusions: Changes in the NI suggested that it may serve as an accurate biomarker of disease progression in ALS. A reduction in NI reflected the loss of peripheral motor units, an expected feature of ALS. The reduction in NI was more substantial than clinical measures of disease progression, including the ALS FRS-r. The NI may be considered for future ALS clinical trials given its high level of responsiveness to disease progression and ease of use.

P172 THE IMPACT OF THE AWAJI-SHIMA CRITERIA ON YIELD FROM CONCENTRIC NEEDLE EMG STUDIES IN PATIENTS WITH SUSPECTED ALS

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Keywords: Awaji-shima, Airlie-House, EMG

Backgound: Amyotrophic lateral sclerosis (ALS) is one of the most debilitating and devastating of the neurological diseases. An early diagnosis is desirable for optimal management and is mandatory for patient enrolment into clinical trials. Clinical neurophysiology can be helpful in establishing a diagnosis in clinically suspected cases. However the Airlie-House criteria have been repeatedly reported to lack the sensitivity required to facilitate early diagnosis. The Awaji-Shima recommendations were formulated in 2006 with the aim of increasing the sensitivity of these diagnostic criteria allowing earlier recognition of the disease. This audit aims to examine how clinical neurophysiology could be employed more effectively to facilitate early diagnosis.

Objectives: To describe the proportion of patients in which the needle EMG studies provided the referring physician with clinically significant additional information. To investigate whether application of the Awaji-Shima recommendations retrospectively to a cohort of patients with suspected ALS improves the yield from the electrophysiological examination and leads to change in the final diagnostic categorisation of the cohort.

Methods: A review was undertaken of the medical records and neurophysiological data of all patients referred to the neurophysiology department at Beaumont Hospital in Dublin with suspected ALS over a 3 year period.

Results: Twenty-nine patients with ALS were identified with a mean age 61.5 (range 37–79 years) and a male to female ratio of 2.2:1. Nineteen patients had spinal onset ALS (64%), 8 had bulbar-onset ALS (28%) and 2 had generalized-onset ALS (8%). Average follow up period was 13.8 months. Nerve conduction studies showed significant motor only abnormalities in 7 patients. EMG studies provided the referring clinician with new clinically significant information in 72% of cases.
The diagnostic category of the patient changed after the EMG study in 42.3% of patients using the Airlie Hose criteria and 69.2% using the Awaji-shima recommendations. The proportion of patients in the probable/definite group was 34.4% prior to the EMG study. This proportion increased after the EMG study to 37.9% when the Airlie House criteria were used and 72.5% using the Awaji-shima criteria.

Discussion and Conclusion: Utilisation of the Awaji-shima recommendations resulted in a significant increase in the proportion of patients in the probable/definite ALS category. The two most common reasons for failure of the EMG study to change the patients diagnostic category were: 1) limited EMG study due to patient intolerance; and 2) presence of widespread chronic innervation in the absence of evidence of active denervation.
This study suggests that the Awaji -shima recommendations increase the yield from concentric needle examination in patients with suspected ALS.

P173 EXPERIENCE WITH THE AWAJI ISLAND MODIFICATIONS TO THE DIAGNOSTIC CRITERIA FOR ALS

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Background: Most cases of amyotrophic lateral sclerosis (ALS) are sporadic, there is no single confirmatory laboratory test and the diagnosis is based on a set of clinical and electrophysiological criteria upon exclusion of other definable diseases. An early and correct diagnosis is important, especially for drug trials aimed at slowing disease progression. To increase sensitivity, an expert group recommended modified electrodiagnostic criteria.

Objectives: To evaluate the performance of the Awaji modified criteria in the electromyography (EMG) laboratory of an ALS referral center.

Methods: We reviewed charts for electrodiagnostic and clinical findings in 70 consecutive patients referred to the EMG laboratory for suspected motor neuron disease over a six-month period to see if they met current or modified ALS electrodiagnostic criteria.

Results: Of the 70 EMG studies included for analysis, 19 did not have neurogenic changes on EMG; 11 studies showed neurogenic changes in 1 region. 40 met the current Revised El-Escorial EMG criteria (EEC-R) (having 2 or more regions with fibrillation/positive sharp waves and neurogenic motor unit potentials), and an additional 3 met the modified Awaji criteria for probable ALS (EEC-A) (having 2 or more regions with fasciculations and chronic motor unit potentials).

Interpretation: In this sample, the modified Awaji criteria showed increased sensitivity. In our center this resulted in 3 additional diagnoses of probable ALS (7%) over a 6-month period. More experience is needed to understand if this modest increase in sensitivity meaningfully improves diagnosis and clinical trial recruitment.

P174 AN EVALUATION OF THE AWAJI SHIMA CRITERIA IN THE DIAGNOSIS OF MOTOR NEURONE DISEASE

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Keywords: El-Escorial Criteria, Awaji-Shima criteria, EMG

Background: New criteria for the diagnosis of Amyotrophic Lateral Sclerosis/Motor Neurone Disease (ALS/MND) were recently proposed at an international symposium in Awaji-Shima, Japan. They differ from the accepted revised El-Escorial criteria by considering fasciculation potentials to be evidence of acute denervation. In addition when assessing diagnostic certainty the Awaji-Shima criteria equate electrodiagnostic evidence of lower motor involvement with clinical examination findings.

Objectives: To establish if the Awaji-Shima criteria enable an earlier diagnosis of MND to be made without increasing the number of false positives.

Methods: A retrospective review was performed of 205 consecutive sets of notes, from patients who underwent neurophysiological assessment for suspected MND during the period 2000–2003. The clinical signs and EMG findings were assessed according to the two sets of criteria. The diagnoses reached using the criteria were compared to the final interval diagnosis.

Results: 107 patients of 205 had a final interval diagnosis of MND. The Awaji Shima criteria had a sensitivity of 60.7% and specificity of 95.9%, compared to the revised El-Escorial criteria with a sensitivity and specificity of 28.0% and 95.9% respectively.

Discussion: The Awaji-Shima criteria increased the sensitivity of diagnosis without affecting the specificity or the false positive rate. Accepting fasciculations as evidence of acute denervation increased the diagnostic certainty of MND. These findings suggest that the Awaji-Shima criteria will enable an earlier diagnosis and potentially earlier recruitment into clinical therapeutic trials.

P175 MUNIX PERFORMED ON BRACHIAL BICEPS MUSCLE WITH QUANTITATIVE AND QUALITATIVE ASSESSMENT OF FORCE

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Keywords: MUNE, MUNIX, voluntary muscle force

Background: Motor unit number estimation (MUNE) is an electrophysiological method designed to quantify motor unit loss in target muscles of interest. This study is a part of multidisciplinary work studying diagnostic features of ALS. MUNE has the potential role of quantifying progression rate in ALS and measuring the response in future treatment trials. This study focuses on a new MUNE method applied on control subjects.

Objectives: To evaluate the ability of a novel MUNE technique, Motor Unit Number Index (MUNIX) (1), to quantitatively assess the number of MUs in a proximal muscle. Thus the purpose of this study was to examine if surface interference pattern (SIP) in MUNIX changed with specified levels of voluntary force. MUSIX is a parameter derived from the test to express motor unit potential (MUP) size.

Methods: Fifteen untrained healthy subjects (7 males, 8 females) – age 27–53 years – with no history of pathology in the upper limb volunteered to participate in the study. In all tests the brachial biceps muscle (BB) of the dominant upper limb was tested. MUNIX was performed with subjective assessment of force. The examiner was blinded for the result in the following test which was planned on a different day. Here the subjects were positioned with 90° flexion at the elbow in dynamometer LIDO Active Multijoint IIa. The computer software package LIDOACT 5.3D was used for data collection. SIP was obtained in 9 recordings (KeyPoint Classic) with gradually increasing voluntary isometric force from slight to maximum activity with force increments of 10% maximal voluntary contraction (MVC). Data analysis with MUNIX. exe was performed offline.

Results: There was a linear relationship between SIP area and force level throughout the test. The relation between number index and force level was similar to that between number index and SIP area. Correlation between number index in BB and CMAP amplitude was high ($p < 0.01$). No relation was found between MUNIX/MUSIX and maximal voluntary contraction.

Discussion and Conclusions: The demonstrated linear relationship between SIP area and force performance supports the hypothesis proposed for MUNIX in which force is substituted by SIP area. In ALS the superimposition among MUPs decreases as the number of MU decreases, thereby theoretically making MUNIX more sensitive for detecting the number of actual MUs.
There is a linear relationship between SIP area and the level of MVC, which is expected according to the mathematical model of MUNIX. MUNIX has the potential to follow the pathophysiological changes in the lower motor system in ALS

patients and a prospective study is ongoing to define more clearly the sensitivity and specificity of MUNE in patients with ALS.

Reference:

1. Nandedkar SD, Nandedkar DS, Barkhaus PE *et al.* IEEE Trans Biomed Eng. 2004; 51(12):2209–11

P176 MOTOR UNIT NUMBER ESTIMATION (MUNE) USING SURFACE EMG INTERFERENCE PATTERN (S-EMG IP) AND COMPOUND MUSCLE ACTION POTENTIAL (CMAP)

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Keywords: Motor Unit Number Estimation, electromyography, compound muscle action potential

Background: Different Motor Unit Number Estimation (MUNE) methods have been described to estimate motor unit (MU) loss in ALS.

Objectives: To develop a MUNE technique (MUNIX) that would be easy and rapid to perform. Using surface EMG recordings, MUNIX could give information on both the estimated number (i.e., "index") of MUs, and also on their size (MUSIX).

Methods: In an 8 month serial study of 10 probable or definite ALS patients and 2 healthy controls, we recorded bilateral hypothenar and thenar CMAPs as obtained in conventional motor nerve conductions. In each instance, the active recording electrode was placed over the muscle to obtain the maximal evoked response. We then made surface EMG interference pattern (S-EMG IP) recordings at different voluntary levels of activation using the same montage to record the CMAP. These signals were analyzed to calculate the Motor Unit Number Index (MUNIX) and Motor Unit Size Index (MUSIX).

Results: Thenar CMAP amplitude decreased by 16% and 24% at 4 and 8 months, respectively. In contrast, MUNIX decreased by 29% and 42% at these two intervals. MUSIX increased by 21% compared to baseline over the 8 month period. Compared to normals however, this represented an almost 78% increase. The hypothenar CMAP declined by 10% and 17% at 4 and 8 months, respectively. MUNIX decreased 21% and 37% at these two interval measures. Hypothenar MUSIX also increased by 21% compared to baseline. But compared to normals, this represented a 91% increase. Each muscle took about 5 minutes to study and could be performed on a standard electromyograph using a special software program.

Discussion and Conclusions: MUNIX and MUSIX provide complimentary information that cannot be derived from the CMAP alone. MUNIX decreased more than the CMAP. MUSIX increased, but not enough to compensate for MU loss. This suggested inadequate compensatory reinnervation. Hypothenar muscle shows greater increase in MUSIX than thenar muscle. This could result in normal CMAP amplitude despite reduced MUNIX. This technique should be of use in studying ALS progression (changes in MUNIX) and reinnervation (changes in MUSIX), where it demonstrates the expected pattern of motor unit (MUNIX) decline as the disease progresses. CMAP amplitude must be controlled in serial investigations.

P177 COMPARISON OF MUNES BY MULTIPLE POINT STIMULATION AND INCREMENTAL STIMULATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: motor unit number estimates, multiple point stimulation, incremental stimulation

Objectives: To compare two common techniques for motor unit number estimation (MUNE), multiple point stimulation and incremental stimulation, in patients with amyotrophic lateral sclerosis (ALS).

Methods: Surface recorded motor unit action potentials of the median nerve/thenar muscle were measured on 60 healthy controls and 60 patients with ALS. The compound muscle action potential (CMAP) amplitude of the maximal baseline to negative peak was recorded. For multiple point stimulation, the stimulus sites included the skin of the wrist, 6 cm above the wrist, elbow, and 6 cm above the elbow. Individual motor unit responses were obtained by moving the stimulating electrode and isolating threshold responses with distinct morphologies. Then, with finely graded stimulus intensity at one point, 3 steps in a CMAP were investigated. 10–12 different single motor unit action potentials (SMUPs) were recorded. For incremental stimulation, stimulus intensity was slowly increased from subthreshold levels until a small, all-or-none response was evoked. The intensity was slowly increased until the response increased in a quantal fashion. This process was repeated for a total of 10 increments. Individual motor unit amplitudes were obtained by subtracting amplitudes of each response from that of the prior response. Both techniques were performed twice, electrodes changed and results averaged.

Results: For healthy controls, MUNE was 227.8 ± 30.2 for multiple point stimulation, 197.7 ± 26.4 for incremental stimulation. Test-retest correlation coefficients and coefficients of variation for the mean of two MUNEs were $(0.88 \sim 0.91)$ and $(13.20 \sim 15.24)\%$ for multiple point stimulation, (0.86) and $(13.30 \sim 15.65)\%$ for incremental stimulation. For ALS patients, MUNE was (63.5 ± 5.7) and (58.6 ± 7.0), respectively.

Conclusions: Both MUNE methods are similar reproducible and are equally effective at documenting progression of a lower motor neuron disorder in patients with ALS.

P178 LOW CMAP AMPLITUDE DECREASES REPRODUCIBILITY OF STATISTICAL MUNE

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Keywords: motor unit number estimation, reproducibility, compound muscle action potential

Background: Reproducibility of motor unit number estimation (MUNE) is of great importance for follow-up studies. Many factors can affect the reproducibility of Statistical MUNE.

Objectives: To evaluate the reproducibility of Statistical MUNE in healthy subjects and amyotrophic lateral sclerosis (ALS).

Methods: Twenty-seven healthy subjects and 29 patients with ALS were recruited. Statistical MUNE was performed in the median nerve/thenar muscle twice in the same day. Number-weighted MUNE (n-MUNE) was calculated. Coefficient of variation (CV) from trial 1 to trial 2 was determined by subtracting the n-MUNE of trial 1 from the n-MUNE of trial 2 and dividing that value by the n-MUNE of trial 1.

Results: In healthy subjects, the n-MUNE from trial 1 and trial 2 were 119.5 ± 9.8 and 120.6 ± 10.6 respectively ($P > 0.05$). In ALS subjects, the n-MUNE from trial 1 and trial 2 were 47.2 ± 23.5 and 44.8 ± 23.0 respectively ($P > 0.05$). $P_{50}(P_{25}, P_{75})$ of CV for n-MUNE were 9.5% (5.6%, 25.0%) in ALS and 5.0% (2.3%, 9.4%) in healthy controls ($Z = 2.387$, $P = 0.017$). In 25% of cases with ALS, CV was more than 25%, and the amplitude of compound muscle action potential in those nerves was from 1.9 mV to 4.3 mV, while in the other 75% patients, CV was less than 25% and the amplitude was from 3.8 mV to 12.4 mV.

Discussion and Conclusions: n-MUNE in healthy subjects and ALS has good test-retest reproducibility. The reproducibility of n-MUNE in ALS is not as good as in healthy subjects. It is recommended that statistical MUNE should be performed in nerves with higher CMAP amplitude for follow-up studies.

P179 MONITORING DISEASE PROGRESSION USING HIGH-DENSITY MOTOR UNIT NUMBER ESTIMATION IN ALS PATIENTS

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Keywords: MUNE, EMG, motor unit number

Background: In ALS the loss of α-motor neurons is masked by collateral reinnervation, so that muscle strength, force measurements and many electrophysiological techniques are unreliable measures of disease progression. Motor Unit Number Estimation (MUNE) is a technique that provides a more direct measure for the amount of motor neuron loss. The number of motor units can be estimated by dividing the compound muscle action potential (CMAP) by a representative motor unit potential (MUP). The representative MUP is usually taken as the mean of single MUPs. In this study we compare the high-density MUNE technique with other measures to determine its usability as a marker of disease progression.

Objectives: To determine disease progression using high-density motor unit number estimation and compare these results with other measures such as the ALS functional rating scale and force.

Methods: High-density surface EMG uses a large number of small densely spaced electrodes over one muscle. Using high-density surface EMG, MUPs can be recognised by their spatial and temporal profiles. The extra information helps to obtain a larger sample of single MUPs. This increases the accuracy and reduces the variability. In a longitudinal study, MUNE measurements were preformed in 18 ALS patients over a period of 8 months. Measurements took place at baseline and at four month intervals. To establish reproducibility, MUNE values were measured twice, at baseline and within two weeks. MUNE values were compared with muscle strength (MRC), functional hand grip strength and thenar pinch strength and the ALS functional rating scale (ALSFRS). Baseline MUNE values were compared with a group of 26 healthy controls.

Results: Mean MUNE for the 26 healthy controls was 280 (range: 131–493). Reproducibility was performed in a subset of 14 subjects (ICC = 0.88). Mean MUNE value at baseline in ALS patients was 152 (range: 13–480). Reproducibility values showed good agreement similar to the healthy group (ICC = 0.87). Longitudinal data showed that MUNE decreased more than CMAP and ALSFRS. In percentage to baseline and over 8 months ALSFRS decreased 14%, MRC 17%, CMAP 36%, functional hand grip 27%, thenar pinch 48%, and MUNE 51%.

Discussion and Conclusions: MUNE decreased most as compared to other measures indicating that it is a sensitive measure to motor neuron loss. Furthermore, high-density MUNE showed good reproducibility in healthy subjects and ALS patients. Pinch force seemed to be sensitive as well but requires a MRC of 4 or higher. We conclude that MUNE should be considered to be used in addition to other markers of disease progression.

P180 MOTOR RESPONSES ELICITED BY DIRECT MECHANICAL STIMULATION OF PERIPHERAL NERVES IN PATIENTS WITH EARLY STAGE ALS

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Keywords: electrophysiology, axonal excitability

Background: Previous studies have demonstrated that ALS patients have widespread disturbances in axonal excitability, probably related to potassium and sodium channel disorders. The evaluation of motor responses elicited by mechanical stimulation (brisk compression or percussion) of peripheral nerves (MREC) could be used as an alternative bedside method to quantify peripheral motor axon excitability. There are no previous studies of MREC in ALS patients.

Objectives: To describe the electrophysiological and clinical features of MREC in early-stage ALS patients.

Methods: Fifteen patients with a diagnosis of definitive ALS (<1 year of evolution) according to the El Escorial criteria and 17 age-matched healthy controls were studied. MREC were elicited using two types of mechanical stimulation to the peroneal nerve at the fibular head: 1) Mechanical compression: briskly sliding the index and middle fingers, transversally, while making pressure over the nerve trunk (like pulling a guitar string); 2) Tapping the nerve trunk with a modified neurologist's reflex hammer that also triggered the electromyographic recordings. Motor responses were recorded with surface electrodes placed over the EDB muscle. The nerve was stimulated 10 times, at intervals of 30 seconds. Peak to peak amplitude and the percentage of occurrence of MREC were measured in all subjects.

Results: The percentage of occurrence was significantly higher in ALS patients than in normal subjects for both types of stimulation: mechanical stimulation ($75.5 \pm 21.3\%$ vs. $25.5 \pm 12.2\%$, $p < 0.009$); percussion: ($35.5 \pm 11.3\%$ vs. $12.5 \pm 5.2\%$, $p < 0.04$). ALS patients showed significantly increased MREC amplitudes, compared to healthy controls, to both variants of stimulation: mechanical compression (1200 ± 250 uv. vs. 900 ± 236 uv., $p < 0.001$) and percussion (650 ± 145 uv. vs. 450 ± 175 uv., $p < 0.001$). No significant correlations were found between the incidence of MREC and the severity of fasciculation potentials, upper motor neuron signs or EMG signs of denervation.

Discussion and Conclusion: These results demonstrate that ALS patients have increased responses to mechanical stimulation of nerve fibers. Direct mechanical compression of the nerve trunk is more effective than percussion for eliciting MREC. The assessment of axonal excitability could be important in the study of the wide spectrum of pathologies involved in motor neuron disease and its differential diagnosis. These findings demonstrate that patients with early stage ALS have increased peripheral motor axon excitability, which can be identified with MREC. The clinical evaluation of MREC, just compressing or percussing the nerve and observing the elicited muscle twitch, is a simple, replicable and widely available tool to test axonal excitability in ALS patients.

P181 NEUROPHYSIOLOGICAL STUDY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN A TOTALLY LOCKED-IN STATE

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Keywords: totally locked-in state, somatosensory evoked potential, electroencephalogram

Background: Fifteen to eighteen percent of amyotrophic lateral sclerosis (ALS) patients present with ophthalmoplegia and fall into a totally locked-in state (TLS) after initiation of tracheostomy positive-pressure ventilation (TPPV).

Objectives: To assess the neurophysiological function in TLS-ALS patients using the techniques of somatosensory evoked potentials (SEP) and electroencephalogram (EEG).

Methods: SEPs were recorded by electrical stimulation of the median nerve at the wrist in seven TLS patients (3 men and 4 women, age: 53 to 81 years). All patients showed complete ophthalmoplegia and tetraplegia with TPPV all day long. Communication with them was entirely impossible. The brain MR images showed severe frontotemporal atrophy in all of the patients. The disease duration ranged from 5 to 13 years and the duration of ventilator use was 3 to 10 years. One patient (66 year-old man) had a superoxide dismutase 1 (SOD1) gene mutation (Exon 5, Cys146Arg). Erb's potential, cervical/brainstem N13 potential (C5S-Fz), parietal N20 (CP3/4-Fz) and frontal N30 (F3/4-A), and central conduction time (latency differences between N13 peak and N20 peak) were analyzed. EEG was recorded from the scalp monopolar electrodes placed on Fp1/2, F3/4, C3/4, P3/4, O3/4, F7/8, T3/4 regions, for about 15 to 30 minutes in the afternoon. The frequency analysis of theEEG was performed for a relatively stable 5 seconds of the total recording time using the technique of Fast Fourier Transform (FFT).

Results: Six out of the seven patients showed decreased or abolished parietal N20 and frontal N30. The latencies of N13 and central conduction time were also delayed. The patient with SOD1 gene mutation showed poor Erb's potentials and no responses of the brainstem and cortical components. In EEG analysis, only two patients showed occipital alpha waves with slow frequency range (8 to 10 Hz), and the other three showed theta to delta waves predominantly on the central-frontal regions. In one patient with involuntary jaw movements, the FFT analysis could not be examined due to EMG artifact. The patients with occipital slow alpha waves showed preserved parietal N20 components, and the patients with no N20 components showed theta to delta range EEG without alpha waves.

Discussion and Conclusions: Along with disease progression of ALS, the sensory system could be involved. The lesions might be in the spinal cord, brainstem and primary sensory cortex. SOD1-associated ALS might involve the peripheral sensory neurons. EEG is desynchronized and slowed in the advanced stages, suggesting impaired consciousness of the patients. There might be a correlation between the presence of N20 in SEP and the result of EEG-FFT analysis. In TLS-ALS patients, communication aids should be applied during the stages with preserved SEPs and alpha rhythms of EEG.

P182 ELECTROPHYSIOLOGICAL PROPERTIES OF LUMBAR MOTONEURONS OF ADULT ANAESTHETIZED MICE: IMPLICATION FOR THE STUDY OF ALS

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Keywords: intracellular recordings, intrinsic properties, adult mouse model

Background: Our understanding of the pathophysiological mechanisms of ALS requires the study of the electrophysiological properties of motoneurons and the mechanical properties of their motor unit in animal models. However, data available so far have been obtained on isolated immature preparations, long before the apparition of any symptoms, or have been focused on the sole contractile properties of muscles. Intracellular motoneuron recordings in mice older than 2 weeks can only be achieved *in vivo*. However, the electrophysiological properties of wild-type (WT) mouse spinal motoneurons have been examined in only a few studies.

Objectives: To present a systematic investigation of the subthreshold and the discharge properties of motoneurons in adult mice.

Methods: We have developed an anaesthetized adult mouse preparation in which we obtained stable intracellular recordings of lumbar motoneurons, and the force of its motor unit. We recorded, in 20 WT mice, 37 motoneurons with a resting membrane potential more hyperpolarized than -50 mV and an overshooting action potential larger than 65 mV.

Results: Three salient features were observed: 1) The membrane time constant was brief (0.5 to 4.0 ms, $2.5 +/ -1.0$ ms, $N = 35$) despite input resistances from 1.2 to

7.1 MOhm (3.6+/−1.7 MΩ, N=37). One consequence is the high frequency of the subthreshold resonance at the resting potential (7–30 Hz, 17+/−6 Hz, N=23). This resonance was likely due to the Ih current. 2) The spikes repolarized quickly (spike width at half height: 0.25–0.50 ms, 0.30+/−0.05 ms, N=25) and the duration (26–67 ms, 45+/−11 ms, N=19) and time constant of the AHP (5–13 ms, 10+/−2 ms, N=19) were short. 3) During depolarizing ramps of current, the motoneurons displayed high frequency oscillations (100–150 Hz) preceding each spike. The fast membrane kinetics favored the appearance of ample oscillations. The interval between two successive spikes was variable depending on the number of oscillations. We called this regime of highly variable discharge the "sub-primary zone". It was observed in all motoneurons that discharged repetitively (N=20). In 15 of them, this zone was followed by a more "classical" primary zone at higher current intensities, on which the gain can be measured meaningfully (5 to 50 Hz/nA, 16+/−10 Hz/nA, N=15). In the primary zone, the AHP dominated the interspike interval and the discharge frequency was much more regular.

Conclusions: This work will serve as a foundation for subsequent works, focused on studying the evolution of the motoneuron's electrical properties, as well as the contractile properties of its motor unit during the development of MND.

P183 ALTERATION IN THE INTRINSIC EXCITABILITY AND UPREGULATION OF THE IONIC CURRENTS OF MUTANT SOD1 MOTONEURONS: *IN-VITRO* RECORDINGS AND COMPUTER SIMULATIONS

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Keywords: electrophysiology, modeling, excitotoxicity

Background: Excitotoxicity is one of the hypotheses for mechanisms contributing to motoneuron degeneration in ALS. Surprisingly, studies of excitotoxicity have largely ignored investigating the changes in voltage-sensitive ion channels, the primary determinant of the motoneuronal excitability.

Objectives: To assess the intrinsic excitability and measure the ionic currents in motoneurons of wild-type (WT) and transgenic mice during the presymptomatic stage, long before disease onset.

Methods: We used two approaches to investigate the excitability of motoneurons: 1) whole cell patch clamp recordings of ionic currents from lumbosacral motoneurons of neonatal (P_0–P_{12}) WT and transgenic mice (G93A model) in the slice preparation; 2) development of realistic computer models of WT and mSOD1 lumbar motoneurons in which ionic currents could be estimated. Models were based on the reconstructed morphologies of neonatal WT and mSOD1 motoneurons (P_8–P_{10}), and model parameters were optimized to match the electrical properties recorded experimentally from the same motoneurons.

Results: The results from the *in vitro* recordings and computer simulations confirmed each other and showed that the intrinsic excitability of mSOD1 motoneurons is altered long before symptom onset. More specifically, the magnitudes of the Na^+ and Ca^{2+} PICs were significantly increased in mSOD1 motoneurons relative to WT. The *in vitro* recordings from the slice preparation indicated that the motoneuronal PICs increased with age and mSOD1 motoneurons P_6 and older had significantly larger PICs than WT littermates (mean±SD: WT PIC 87±99 pA, n=33; mSOD1 PIC 152±147 pA, n=40). Tetrodotoxin (Na^+ channel blocker) and isradipine (L-type Ca^{2+} channel blocker) were applied to quantify the Na^+ and Ca^{2+} contributions to the PIC. Na^+ currents mediated a larger portion of the PIC than Ca^{2+} and were significantly larger in mSOD1 motoneurons over P_6 (Na^+ PIC: WT 199±100 pA, n=14; mSOD1 336±262 pA, n=23). In accordance with the *in vitro* recordings, computer models of mSOD1 motoneurons exhibited an increase in the magnitudes of the Na^+ and Ca^{2+} PICs relative to WT models. For instance, mSOD1 motoneuron models predicted an increase in the total, Na^+, and Ca^{2+} PICs by 45%, 55%, and 40% relative to WT models, respectively. Experimentally, these percent increases in PIC amplitudes were comparable and 80%, 60% and 50% were measured in mSOD1 motoneurons, respectively. Computer simulations also predicted alteration in the membrane biophysical properties of mSOD1 motoneurons.

Discussion and Conclusions: Spinal motoneurons that are vulnerable in ALS have low Ca^{2+} buffering capacity. Increases in Ca^{2+} entry to these cells, through Ca^{2+} channels or the prolonged depolarization induced by Na^+ channel activation, could contribute to their degeneration. These results indicate that upregulated ionic mechanisms in mSOD1 motoneurons may contribute to their degeneration in ALS.

P184 PERIPHERAL NERVE PATHOLOGY IN CANINE DEGENERATIVE MYELOPATHY WITH MUTATION IN SUPEROXIDE DISMUTASE 1 GENE

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Keywords: peripheral neuropathy, dog, lower motor neuron

Background: Canine degenerative myelopathy (DM) is a progressive neurodegenerative disease which has recently been shown in several canine breeds to be a result of a missense mutation in the superoxide dismutase 1 (*SOD1*) protein (1). Based on this mutation and similarities in disease phenotype, canine DM appears to be a spontaneously occurring model for human amyotrophic lateral sclerosis (ALS). In canine DM, upper motor neuron spasticity and general proprioceptive pelvic limb ataxia occurs in dogs older than 8 years of age and if euthanasia is delayed, clinical signs will ascend causing flaccid tetraparesis and other lower motor neuron signs. A similar course of clinical progression has been described in upper motor neuron onset human ALS.

Objectives: To determine if pathologic changes typical of denervation are present in muscle (biceps femoris and gastrocnemius), and nerve (peroneal) of dogs with advanced DM.

Methods: Specimens were collected either as biopsies or following euthanasia from Pembroke Welsh Corgi (8), Boxer (7), German Shepherd Dog (2), Kerry Blue Terrier (1) and Chesapeake Bay Retriever (1) dogs homozygous for the *SOD1* mutation and confirmed as DM with microscopic demonstration of DM-specific lesions in spinal cords. Muscle specimens were evaluated in both frozen and paraffin sections and peripheral nerve specimens were evaluated in resin sections. Abnormalities were generally classified as denervation atrophy, nerve fiber loss and axonal degeneration or demyelination, and were scored 0 to +++ (normal to marked, respectively). Similar specimens were collected and processed from age-

matched control dogs including Boxer (3), German Shepherd Dog (1), Rhodesian Ridgeback (3) and English Cocker Spaniel (1) shown to be clear or heterozygous for the mutation.

Results: The most dramatic and consistent abnormalities were found in the Pembroke Welsh Corgi in which denervation atrophy (+++) was present in all muscle specimens and nerve fiber loss (++ to +++), myelin ovoids (++), myelin splitting and ballooning (++ to +++) and inappropriately thinly myelinated fibers (++ to +++) consistent with mixed axonal degeneration and demyelination were found in peroneal nerves. Similar but milder changes (+ to ++) were present in peripheral nerves in the Boxer, German Shepherd Dog, Kerry Blue Terrier and Chesapeake Bay Retriever breeds with variable muscle atrophy (0 to ++).

Discussion and Conclusions: Longer disease duration in the Pembroke Welsh Corgi (24 to 48 months) versus other affected breeds (7 to 12 months) may explain the more extensive disease severity. In conclusion, this study provides pathologic evidence for peripheral nerve involvement in canine DM consistent with the clinical signs of lower motor neuron disease.

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P185 DETERMINANTS OF DOUBLE DISCHARGES IN AMYOTROPHIC LATERAL SCLEROSIS AND KENNEDY DISEASE

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Keywords: double discharge, Kennedy disease, transcranial magnetic stimulation (TMS)

Background: Motor unit double discharges (DDs) occur frequently in motor neuron disorders such as Amyotrophic Lateral Sclerosis (ALS) and Kennedy disease (KD). While this is likely to be because of changes in the intrinsic properties of motor neurons in such disorders, changes in corticomotoneuronal inputs may also contribute.

Objectives: To assess the contribution of corticomotoneuronal inputs to DDs.

Methods: The prevalence and intra-doublet interval (IDI) of motor unit DDs as well as their timing with respect to transcranial magnetic stimulation (TMS)-induced primary peaks (PPs) in the peristimulus time histogram (PSTH) were studied in 23 ALS patients (96 motor units), 11 patients with KD (45 motor units) and 13 control subjects (60 motor units). Three DD patterns with respect to the PP were defined: pre-peak DD, peak-DD and post-suppression DD.

Results: In patients with KD more motor units (82%) fired DDs than in ALS patients (51%) and control subjects (63%) ($p = 0.013$). The motor units in patients with KD exhibited the largest motor unit action potential (MUAP) amplitude and shortest interspike interval (ISI). The prevalence of pre-peak DD in Kennedy patients was 4.06 fold higher (95% CI 0.53–2.81; $p = 0.0014$) than in controls whereas in ALS patients the prevalence of peak DD was 4.79 fold higher (95% CI 1.09–21.10; $p = 0.041$) than in controls. Only in ALS the pre-peak IDI (17.1 ± 5.4 ms) and peak IDI (14.4 ± 5.3 ms) were significantly prolonged ($p < 0.003$) compared with controls (12.3 ± 5.3, 6.2 ± 4.1 respectively). MUAP amplitude ($r = 0.344$; $p < 0.005$), estimated amplitude of the excitatory postsynaptic potential (EPSP), ($r = 0.483$; $p < 0.001$) and ISI ($r = 0.456$; $p < 0.001$) were significantly correlated with pre-peak DD prevalence but not with the prevalence of peak DD.

Discussion and Conclusions: The high peak DD prevalence and prolonged IDIs in ALS are consistent with complex and multiple corticomotoneuronal volleys indicating that in ALS upper motor neuron abnormalities also contribute to the generation of double discharges. In ALS and KD MUAP amplitude, ISI and EPSP size reflecting motor unit remodelling and associated changes of intrinsic anterior horn cell membrane properties determine the prevalence of DD.

P186 TRIGEMINO-CERVICAL RESPONSE IN PATIENTS WITH X-LINKED SPINAL AND BULBAR MUSCULAR ATROPHY

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Keywords: trigemino-cervical response, X-linked spinal and bulbar muscular atrophy, lower brainstem

Objectives: The trigemino-cervical response (TCR) was investigated in patients with X-linked spinal and bulbar muscular atrophy (SBMA) to evaluate its effect for disclosing the bulbar involvement in this disorder.

Methods: We studied 30 normal subjects, 30 patients with amyotrophic lateral sclerosis (ALS) and 30 patients with SBMA. In all normal subjects, stimulation of the infraorbital nerve on one side produced bilateral short latency waves, which consisted of a positive/negative wave described with the mean peak latency (P19/N31). The mean square root of the ratio between the amplitude of P19/N31 and the mean rectified surface EMG activity preceding the stimulus was described by A value.

Results: The latency of ipsilateral P19 in SBMA patients was 24.34 ± 4.82 ms, N31 was 36.20 ± 4.91 ms, and the A value was 1.24 ± 0.33, respectively. At the same time, the contralateral P19, N31 and A value in SBMA patients were 23.91 ± 4.84 ms, 35.45 ± 4.76 ms and 1.19 ± 0.25, respectively. The parameters of TCR between SBMA patients and the healthy controls were statistically different ($P < 0.01$). Although there was no statistical difference in the latencies of TCR between patients with SBMA and ALS, the A value was statistically different between these two groups.

Conclusions: TCR can be helpful in disclosing lower brainstem lesions in SBMA patients.

P187 SLOW SACCADES IN AMYOTROPHIC LATERAL SCLEROSIS: A PSP VARIANT?

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Keywords: saccades, bulbar-onset, progressive supranuclear palsy

Background: Classical teaching is that eye movements are spared in amyotrophic lateral sclerosis (ALS), except in those patients whose lives are prolonged by artificial ventilation. Over the past twenty years, however, a number of papers have reported that ALS patients may show a range of eye movement disorders similar to those encountered in other degenerative and hereditary neurological diseases. In particular, numerous case reports describe slow saccades and supranuclear vertical gaze palsies similar to that found in patients with Progressive Supranuclear Palsy (PSP) particularly in bulbar-onset patients. In addition, extrapyramidal features are occasionally found in ALS patients.

Objectives: To examine reflexive saccades in PSP and ALS patients, in particular bulbar-onset patients compared to controls. To compare results between ALS and PSP patients.

Methods: Eye movements were recorded in 44 ALS patients (14 bulbar-onset and 30 spinal-onset), 7 PSP patients and 45 age-matched controls using infra-red oculography. Saccadic speed and latency were measured for horizontal saccades.

Results: Saccades were similar between ALS patients and controls. However, bulbar-onset patients had slower saccades compared to controls ($p = 0.3$) as did PSP patients compared to controls ($p = 0.1$). Saccades were similar between bulbar-onset ALS and PSP patients for speed and latency.

Discussion: The authors propose that slow saccades occur in ALS, particularly in those with significant bulbar disease. Saccades may be similar in bulbar-onset ALS and PSP. This study, however, is limited by the examination of only horizontal saccades as vertical saccades tend to be involved earlier and more severely in PSP. In bulbar-onset disease, however, it is likely that more extensive pathological changes in the brainstem contribute to slowed saccades with or without a supranuclear gaze palsy. In these instances ocular motor abnormalities may imitate those of PSP. Clinicians should therefore be aware of the potential for confusion due to the overlap between the ocular motor findings in both conditions.

P188 USEFULNESS OF OROPHARYNGOESOPHAGEAL SCINTIGRAPHY FOR EVALUATING TRACHEO-BRONCHIAL ASPIRATION IN NEUROLOGICAL PATIENTS

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Keywords: aspiration, scintigraphy, dysphagia

Objectives: Dysphagia and bolus aspiration are two of the most frequent and debilitating symptoms of various neurological diseases. Swallowing disturbances are the most important cause of tracheobronchial inhalation, which causes pneumonia ab-ingestis, whose mortality rate ranges from 20% to 62%. Oropharyngoesophageal scintigraphy (OPES) permits functional quantitative assessment of the various stages of swallowing, and also reveals and quantifies the presence of bolus aspiration. In this study, we employed OPES to dynamically evaluate deglutition in neurological patients (Amyotrophic Lateral Sclerosis, Parkinson's disease, stroke, etc.) to diagnose and quantify bolus aspiration.

Methods: We enrolled 29 neurological patients complaining of dysphagia (16 women and 13 men, mean age 68.2 ± 12.1 years). All patients underwent OPES with ^{99m}Tc-nanocolloid using both a liquid and a semi-solid bolus sequentially. The following parameters were analysed: Oral, Pharyngeal and Esophageal Transit Time, Oro-Pharyngeal Retention Index, Esophageal Emptying Rate, and Aspiration Rate (AR). AR was calculated as: $AR = (IA/AT_0 - AT_1)/100$, where IA stands for Inhaled Activity, while AT_0 and AT_1 stand for Oral Activity before and after swallowing the radioactive bolus, respectively.

Results: OPES revealed tracheobronchial inhalation in 22/29 patients. In particular, 5 patients showed laryngeal aspiration with a mean 7% AR fraction, 10 patients showed tracheal aspiration (mean 18% AR) and the remaining 6 patients had bilateral broncho-pulmonary aspiration (mean 45% AR mean). In one patient with massive bilateral broncho-pulmonary aspiration caused by post-radiotherapy damage of cranial nerves, OPES indicated functional laryngectomy as the most appropriate surgical treatment.

Conclusions: OPES allows an objective, quantitative evaluation of bolus inhalation into the tracheobronchial tract. It is easy to perform, well tolerated by patients, repeatable, and inexpensive. Although the resolution of this technique is too low to clearly define anatomic structures, OPES is useful for determining the exact fraction of the inhaled bolus into the tracheobronchial tree in neurological patients with dysphagia and represents a useful and accurate tool to monitor response to medical and/or surgical therapy.

P189 *IN VIVO* MEASUREMENT OF THE ELASTIC PROPERTIES OF THE SKIN AS A BIOMARKER FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: biomarker, skin elasticity, in vivo study

Background: CNS and skin are both derived from the neural crest and many diseases affect both systems. Published clinical studies in patients with ALS report that decubitus ulcers are

uncommon in immobile patients and that skin is supple with reduced elasticity. Various structural and biochemical changes in skin biopsies occur, including: decreased collagen bundles with amorphous material between them (1) and a non-inflammatory vasculopathy and deposits of beta-amyloid protein near blood vessels and epidermal appendages (2). Significant negative correlation between the diameter of collagen fibrils in the skin and the duration of disease is also reported (1). The biomechanical properties of the skin reflect the complex interplay among epidermis, dermal collagen and elastin networks and underlying structures. The infrequency of decubitus ulcers and the reduced skin elasticity in ALS patients could result from multiple biochemical and structural changes mirroring those in the nervous system.

Objectives: To test the hypothesis that longitudinal quantitative measurements of skin elasticity may be a useful non-invasive biomechanical biomarker of disease progression and a diagnostic aid in patients with ALS.

Methods: We quantitatively evaluated multiple components of skin elasticity in patients with ALS and control individuals using the Cutometer®, a device that measures non-invasively elements of skin elasticity including elastic recovery after release of maximum negative pressure and elastic displacement from initial position. Forty-one consecutively available patients with ALS (based on the El Escorial Criteria) and thirty-one family members (controls) were enrolled and evaluated at baseline. 37 subjects and 26 controls were evaluated at 3 months and 32 subjects and 24 controls at 6 months with Cutometer® measurements of the arm and back. Neurological variables including the ALSFRS-R and FVC were also assessed in patients.

Results: Skin elasticity was significantly reduced in the ALS arm readings ($p < 0.001$) at baseline, indicating that patients with ALS have diminished skin elasticity as compared to unaffected control individuals. Back skin elasticity in ALS patients was significantly correlated with the ALSFRS-R ($p < 0.01$) over time. The elasticity of the back skin in ALS was significantly correlated with the FVC ($p < 0.05$) over time (3 months follow up data analysis shown and six month data analysis will be available at the conference).

Discussion and Conclusions: Skin elasticity is readily measured quantitatively and non-invasively and appears to be a promising biomarker for detecting the ALS phenotype and predicting disease progression. Further studies are needed to elucidate the relationship of this biomarker to specific biochemical changes relevant to the pathogenesis of ALS.

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P190 THE SKIN AS A MIRROR OF ALS PATHOLOGY: MATRIX METALLOPROTEINASES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: matrix metalloproteinase, skin, cerebrospinal fluid

Background: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease that mainly affects the cortical and spinal motor neurons but may also include other organs such as the skin. Matrix metalloproteinases (MMP) have been suggested to play an important role in ALS pathology.

Objectives: To determine whether gelatinase MMP-2 and MMP-9 could provide a link between neuronal degeneration and skin alterations observed in ALS and whether they were related to markers of oxidative stress.

Methods: We measured CSF, serum and skin tissue homogenate concentrations of MMP-2 and MMP-9 using ELISA and malondialdehyde (MDA), an established marker of lipid peroxidation, using High Performance Liquid Chromatography (HPLC) in 54 ALS patients and 36 controls.

Results: We found CSF and skin MMP-9 concentrations to be elevated in ALS patients as compared to controls ($p < 0.001$, $p = 0.03$, respectively). We observed the elevation of MMP-9 in CSF to be independent of the stage of disease or clinical subtypes and to be highest in patients with a rapid progressive course of disease ($p = 0.008$). In contrast, we found no significant difference of CSF, serum or skin concentrations of MMP-2 as compared to controls. We observed CSF MMP-2 to decrease with duration of disease ($p = 0.04$, $R = -0.31$). MDA was elevated in serum of ALS ($p < 0.001$), though no correlation with MMP-2 or MMP-9 was observed.
We observed MMP-9 to be significantly elevated in the spinal cord of SOD1 as compared to WT mice at an age of 60 days ($p = 0.03$), 90 days ($p = 0.03$) and 120 days ($p = 0.01$). We furthermore found MMP-9 to be significantly elevated in skin tissue homogenate of SOD1 as compared to WT at an age of 60 days ($p = 0.04$) and 90 days ($p = 0.04$). We observed a significant correlation of skin and spinal cord MMP-9 concentrations in SOD1 at an age of 30 days ($p = 0.04$) and for all time points combined ($p = 0.04$).

Discussion and Conclusions: These results indicate a general upregulation of MMP-9 in ALS. This may mirror ongoing neuronal degeneration and glial activation and could be linked to alterations in collagen metabolism observed in the skin of ALS patients. MMP-2 seems to be less responsive to disease associated stimuli than MMP-9, though it is functionally related to it. This may be due to differences regarding the regulation of MMP gene expression.
MMP-9 could provide a link between neuronal degeneration and skin pathology in ALS. The skin may provide an easily accessible source of *in vivo* biomarkers in ALS.

P191 PERIPHERAL OXIDATIVE STRESS BIOMARKERS IN SPORADIC ALS AT THE ONSET

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Keywords: oxidative stress, biomarkers, redox imbalance

Background: Among the pathogenic hypotheses on motor neuron degeneration leading to amyotrophic lateral sclerosis (ALS), the reactive oxygen species generation and oxidative stress theory has been put forward. Evidence of accumulation of oxidative damage to proteins, lipids, and DNA in ALS patients has been reported. However, the exact role of oxidative stress at the onset or at a very early stage of the disease is still unclear.

Objectives: To assess oxidative stress biomarker levels in sporadic ALS patients at the onset of disease.

Methods: Oxidative stress biomarkers (advanced oxidation protein products (AOPP) and ferric reducing antioxidant power (FRAP)) were examined in blood samples from 32 sporadic ALS (sALS) patients (13/19 M/F, mean age 63.3 ± 10.8 years) at the onset of the disease (mean time between onset of the symptoms and diagnosis with collection of the blood sample 5.7 ± 2.2 months). A group of 54 healthy matched subjects (25/29 M/F, mean age 69.3 ± 9.2 years) were recruited as controls. The determination of AOPP and FRAP levels was based on spectrophotometric detection.

Results: Plasma AOPP levels were found to be increased in sALS patients compared to controls (346.409 ± 40.1, 246.9 ± 40.9 nmol/microliter, $p < 0.01$). After stratification by gender, the association was significant in the female group (393.6 ± 41.9 vs 238.6 ± 42.5, $p < 0.05$), but not for the male group (316.5 ± 38.9 vs 256.5 ± 34.6, $p = 0.21$). Plasma FRAP levels were found to be decreased in sALS patients compared to controls (0,696 ± 0.057 vs 1.314 ± 0.09 nmol/microliter, $p < 0.001$). After stratification by gender, we observed that plasma FRAP levels were significantly decreased in both males (0.706 ± 0.06, $p < 0.001$) and females (0.688 ± 0.09, $p < 0.05$) compared to controls (1.514 ± 0.06 nmol/microliter). No correlation between AOPP or FRAP values and site of onset of the disease was observed.

Conclusions: These findings support a role for oxidative stress in ALS, even in a very early phase of the disease. FRAP and AOPP can represent useful biomarkers to detect redox imbalance in such disease, thus providing a non invasive tool to monitor disease status and response to therapies.

P192 THE INDIRECT INVOLVEMENT OF RADICAL PROCESSES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: tryptophan derivatives, nitric oxide, fatty acids

Background: Accumulating data indicate that oxidative and radical stress plays a major role in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). It causes damage to main cellular components such as lipids, proteins and nucleic acids, resulting in cell death by necrosis, apoptosis, or autophagy. In our laboratory, we have indirectly evaluated these radical processes by identifying specific immunoglobulins of G, M and A isotypes, directed against neoantigens resulting either from hidden self antigens or self antigens modified by radical species. Here, we reported the binding of circulating antibodies in ALS patient sera, directed against: tryptophan derivative (TD) conjugates, NO and NO_2-modified amino acid conjugates, fatty acids (FA) and their hydroxylated (OHFA) form conjugates.

Objectives: The first objective of this study was to define the specific circulating antibodies present in ALS patient sera directed against TD conjugates, NO and NO_2-modified amino acid conjugates and FA and OHFA conjugates. The second objective was to determine their interest for a better understanding of the diversity of radical processes.

Methods: The TD antigens kynurenin, 3OH-kynurenin and kynurenic, picolinic, xanthurenic, anthranilic, 3OH-anthranilic, quinolinic, quinaldic acids, were each linked to bovine serum albumin (BSA) using carbodimide coupling reaction. NO- and NO_2-conjugates resulted from the following compounds: nitrotyrosine, tyrosine, phenylalanine, citrulline, tryptophan, asparagine, creatin, cysteine, methionine, histidine, arginine, linked to BSA via glutaraldehyde reaction and then nitrosylated. We have used hydroxylated or non-hydroxylated caproic, caprylic, capric, lauric acids; and myristic, palmitic, oleic and azelaic acids. FA and OHFA were linked to BSA using ethylchloroformiate coupling reaction. Using ELISA tests and these conjugates, the circulating antibodies were identified.

Results: These studies have shown that: 1) specific circulating antibodies directed against TD, NO- and NO_2-modified amino acid conjugates and OHFA components were found with a specific pattern; 2) specific isotype profiles were reported: IgA responses were directed only against kynurenin, kynurenic, quinolinic, quinaldic, and 3OH-anthranilic conjugates. We have found a preferential IgG response to NO-tryptophan, IgM response to NO-phenylalanine, NO-tryptophan and NO-BSA, and IgA to NO-tryptophan and 3 isotypes for nitrotyrosine. Only IgM responses to hydroxylated caproic acid were found.

Discussion and Conclusions: These data indirectly confirm that neurotoxic TD, NO and NO_2-modified amino acid conjugates are implicated in neurodegenerative mechanisms. The presence of TD, NO, NO_2^- and OHFA modified endogenous epitopes confirms the alterations in protein structures that could lead to modification or inhibition of enzyme and mitochondrial activities. Subsequently, the latter are able to induce apoptotic and autophagic processes involved in the pathogenesis of ALS. These data indirectly show the diversity of radical processes in ALS.

P193 INVESTIGATION OF LEVELS OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR IN CEREBROSPINAL FLUID IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: VEGF, CSF, hypoxia

Background: Deletion of the hypoxia-response element in the Vascular Endothelial Growth Factor (VEGF) promotor causes adult-onset progressive motor neuron degeneration in mice. Neuroprotective and neurotrophic properties of the vascular endothelial growth factor were revealed in recent studies. These results suppose that VEGF participates in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS).

Objectives: To measure levels of VEGF in the cerebrospinal fluid (CSF) of ALS patients.

Methods: The study concerned 24 ALS patients and 11 control subjects (traumatic patients without craniocerebral

injuries).VEGF was measured by enzyme-linked immunosorbent assay. Spirometry was performed in all ALS patients. Percentage of predicted lung vital capacity was calculated for every patient.

Results: The results have shown that VEGF levels in CSF are significantly decreased in patients under 40 years old compared with controls and with patients over 40 years old. There were significant negative correlations between VEGF levels and hematocrit and between VEGF levels and ALS Functional Rating Scale. There were significant positive correlations between VEGF levels and percentage of predicted Lung Vital Capacity (only in a subgroup of patients who had less than 85% of predicted Vital Capacity) and between VEGF levels and blood pH (only in a subgroup of ALS patients over 40 years old).

Discussion and Conclusions: VEGF is a hypoxia inducible factor. If the VEGF level increases in hypoxia it should have the following correlations: positive correlation with hematocrit, negative correlation with ALS Functional Rating Scale, negative correlations with predicted percentage of Lung Vital Capacity and blood pH. Inverse correlations suppose a disorder of hypoxia inducible factors in ALS patients.
Distinction of VEGF levels in the CSF of patients less than forty years of age is evidence of some heterogeneity of disease and may be a mark of difference in pathogenesis of ALS in adult and middle aged patients. Revealed correlations of CSF VEGF levels with some laboratorial indexes suggest an inadequate hypoxia reaction in ALS patients. Further investigations could help to assess the relevance of this inadequate hypoxia reaction in the pathogenesis of ALS.

P194 CORRELATION OF CEREBROSPINAL FLUID GLIAL MARKERS WITH SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: biomarker, cerebrospinal fluid, glial activation

Background: Biochemical markers in the cerebrospinal fluid (CSF) are increasingly studied in amyotrophic lateral sclerosis (ALS) to evaluate their relevance for differential diagnosis, disease progression, and for the understanding of pathophysiological processes. We therefore evaluated glial proteins in the CSF of patients with ALS and other motor neuron diseases (MND) in order to assess whether baseline levels of CSF measures in ALS are associated with the course of the disease.

Methods: 108 subjects with MND were included (ALS, n = 90; lower motor neuron diseases such as spinal muscular atrophy, n = 12; upper motor neuron diseases, n = 6). Follow-up data were available in 23 ALS patients. CSF baseline levels of astroglial S100beta (all MND patients) and microglial sCD14 concentrations in CSF and serum (57 ALS patients, 13 patients with other MND and 28 controls) were related to diagnosis, duration of the diseases, clinical scores at time of lumbar puncture and survival.

Results: Compared with lower motor neuron diseases, CSF S100beta levels were higher in ALS ($p = 0.002$) and upper motor neuron diseases ($p = 0.004$). In ALS patients, CSF S100beta concentrations showed an inverse correlation with survival ($r = -0.457$, $p = 0.011$, $n = 23$). CSF/serum ratios of sCD14 were decreased in ALS and lower motor neuron diseases as compared to controls ($p < 0.001$). CSF sCD14 positively correlated with the survival time ($r = 0.643$, $p = 0.009$, $n = 13$). There were no correlations of CSF baseline levels of both glial markers with clinical scores such as the ALSFRS or muscular strength, or the duration of the diseases.

Conclusions: Although CSF S100beta and sCD14 concentrations were not related to clinical scores at time of lumbar puncture, biochemical markers may provide important information about the condition of glial cells and therefore may be useful for prognostic purposes in ALS.

P195 CORRELATIONS BETWEEN PATHOLOGY AND GENE EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: neurodegeneration, biomarker

Background: Amyotrophic lateral sclerosis (ALS) is poorly understood at the molecular level and there are as yet no effective therapeutics to stop the insidious progression of weakness that ultimately leads to respiratory failure and death. The degree of neuronal loss in the spinal cord appears to be related to the degree of clinical severity of ALS (1). Interestingly, motor neuron degeneration generally begins focally in one region of the spinal cord and sequentially works its way to adjacent regions of the nervous system (2) suggesting that a local comparison of different segments of the spinal cord or brain with different degrees of neuronal loss may offer clues to the molecular pathogenesis of disease progression.

Objectives: To compare gene expression in the spinal cords and brains of patients with ALS in regions with differing degrees of neuronal loss and compare this to controls.

Methods: Pathological changes were measured histologically for motor neuron numbers, demyelination, and gliosis; and gene expression was determined by quantitative PCR for a number of genes implicated in the disease process.

Results: Regions of the spinal cords and brains of patients with ALS disease showed differing degrees of neuronal loss and activation/inflammation of non-neuronal cells. While there was no consistent change in SOD1, TDP43, and EAAT2 expression (genes implicated in pathogenesis), there was a significant downregulation of NRG1 gene expression (a neuronally-expressed gliotrophic gene that supports axoglial interactions). On the other hand, the neurotrophin BDNF appeared to have the opposite pattern of expression to NRG1 in the ALS patients. These results suggest that motor neuron loss is correlated with decreased NRG1 but increased BDNF gene expression in ALS patients.

Discussion and Conclusions: The downregulation of NRG1 with upregulation of BDNF could help define altered cell-cell signal patterns in ALS and could serve as a biomarker for measuring neuronal loss in this disease.
Recently, we have postulated a feedback loop between NRG1 and BDNF as a mediator for both axoglial and neuromuscular stability. This raises an intriguing hypothesis that this feedback loop could be defective in ALS and that NRG1

deficiency could be an important biomarker for neuronal loss. This work also sets the stage for higher throughput studies that can identify disruptions in normal regulatory networks that contribute to disease progression and could represent important new biomarkers and therapeutic targets for ALS.

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THEME 8 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P196 PREVALENCE AND DISTRIBUTION OF FASCICULATIONS IN HEALTHY ADULTS: EFFECT OF AGE, CAFFEINE CONSUMPTION AND EXERCISE

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Keywords: fasciculations, ultrasonography

Background: Fasciculations are not always pathological and can occur in healthy individuals.

Objective: To study the prevalence and distribution of fasciculations in healthy adults and assess the effect of age, caffeine and exercise.

Methods: Fasciculations were studied with ultrasonography in 58 healthy adults in various age categories. Questionnaires were used to determine effect of caffeine and regular exercise on the presence of fasciculations. Finally, we tested the effect of strenuous exercise on fasciculations in 10 healthy adults.

Results: Twenty-five subjects (43%) showed fasciculations on ultrasonography, mostly in the abductor hallucis longus muscle. Fasciculations were only sporadically encountered in muscle groups above the knee. Subjects with fasciculations were significantly older than those without. Caffeine and regular physical exercise did not influence the prevalence of fasciculations. However, strenuous physical exercise caused a temporary increase in fasciculations, but only in lower leg muscles.

Conclusion: Fasciculations above the knee should raise suspicion and may warrant further investigation.

P197 PROSPECTIVE COHORT STUDY OF HYPERLIPIDEMIA IN ALS PATIENTS

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Keywords: hyperlipidemia, ALSFRS-R Delta FS, survival

Background: Hyperlipidemia is reported to be associated with an improved survival in ALS. These findings are supported by earlier studies which reported a poor survival and quality of life in malnourished, underweight patients and improved clinical outcomes in association with enhanced lipid and carbohydrate diet in the SOD1 mouse model.

Objectives: To determine prospectively the relationship between fasting lipid profiles at time of diagnosis with rate of progression as a predictor of survival in ALS patients.

Methods: Fasting lipid profiles were collected from patients diagnosed with probable, definite ALS from November 2008 to March 2009. The rate of progression prior to diagnosis was measured as the ALSFRS DeltaFS, a predictor of survival. Subjects were stratified for slow (<0.5 points (pts)/month), medium (>0.5–1.0 pts/month), or fast disease progression (>1.0 pts/month). Average total cholesterol, triglycerides, HDL, LDL levels and LDL/HDL ratios were compared between these groups with Students t-test. $P<0.05$. Change in ALFRS, % FVC, MIP and BMI were analyzed in stratified subgroups of follow up patients.

Results: Sixty-three patients with probable, definite ALS were enrolled in the study. One third of patients were stratified in each group based on progression (fast: $0.31+/-0.12$; moderate: $0.79+/-0.14$; slow: $1.6+/-0.75$). Fast progressing patients were significantly older than the other subgroups (fast: $65.1+/-3.4$ vs. moderate: $57.7+/-15.7$ & slow: $57.8+/-13.9$; $p=0.045$) and had shorter disease duration (fast: $10+/-6.8$ vs. moderate: $16.2+/-8.3$ & slow: $24.6+/-18$; $p=0.007$). Although there was no difference in lipid levels between groups, slow progressing patients showed a trend toward higher LDL/HDL ($p=0.09$ slow vs. moderate) and LDL levels ($p=0.13$ slow vs. fast). Forty-six percent ($n=29$) followed up over 6 ± 3 months. There was no difference in actual rate of decline for ALSFRS-R, % FVC, maximal inspiratory pressure (MIP), or BMI between stratified groups using DeltaFS.

Discussion and Conclusion: This pilot study suggests that patients can be stratified at time of diagnosis based upon estimated rate of progression utilizing the DeltaFS. Furthermore, slow progressing patients show a trend toward elevated LDL and LDL/HDL. A multicenter USA study with a larger sample size is currently being planned to determine the relationship between lipid levels and survival and to further validate the Delta FS as a predictor of survival. Additional questions include the correlation of rate of progression, change in BMI, and respiratory status with change in lipid levels.

P198 THE CORRELATION OF CAGS REPEAT SIZE WITH THE ONSET AGE IN 30 CHINESE PATIENTS WITH KENNEDY'S DISEASE

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Keywords: Kennedy's disease, CAG repeated sequence, Appel ALS score

Objectives: To investigate the correlation of CAGs repeat size and the onset age in Chinese patients with Kennedy's Disease (KD).

Methods: We detected the number of CAG repeats in the androgen receptor genes in 30 patients with KD. The correlation of CAGs repeat size with the onset age was analyzed. At the same time, the Appel ALS score that could represent the degree of motor functional impairment was

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scored in every patient. The correlation of Appel ALS score with the CAGs repeat size and the course of disease were analyzed.

Results: Significant correlation was found between the number of CAGs and the onset age ($r = -0.671$, $P < 0.01$). There was also correlation between the Appel ALS score and the course of disease ($r = 0.855$, $P < 0.01$), but no correlation between the Appel ALS score and the number of CAGs ($r = 0.100$, $P = 0.601$).

Conclusions: As for other CAG repeat diseases, the length of the polyglutamine tract determines the patient's onset age but has no correlation with the pathogenetic condition.

P199 A FOLLOW-UP STUDY FOR SPINAL SURGERY AFTER THE ONSET OF SYMPTOMS OF ALS

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Keywords: cervical spondylotic myelopathy, spinal surgery, prognosis

Background and Objectives: Distinguishing between cervical spondylotic myelopathy (CSM) and ALS can be difficult, because of their similar clinical manifestations. Sometimes, CSM may coexist with ALS since both diseases preferentially affect individuals of middle or old age. Attempts to differentiate between CSM and ALS must persist until diagnosis of one (or both) of the disorders is made, since both the prognosis and treatment of the two are different. Choice of surgical treatment remains controversial. We investigated 19 patients with ALS to clarify the complications of cervical spondylosis and to explore the influence of surgical treatment for their prognosis in a retrospective study.

Methods: A total of 329 consecutive patents with definite or probable ALS were enrolled. The clinical features, especially the history of spinal surgical treatment, were recorded. The patients were followed up every 3 months from visit to death or tracheostomy.

Results: Of 329 sporadic ALS cases, we found cervical spondylosis in 156 patients (47.4%); 19 (5.8%) of them underwent spinal surgical treatment in the early stage of the course of their ALS. No differences between ALS patients who underwent spinal surgery and other ALS patients were noted regarding age at symptom onset, gender and ALSFRS-R at time of diagnosis ($P > 0.05$). However, the time from symptom onset to diagnosis was significantly longer in patients with spinal surgery ($P < 0.001$). In the follow-up study, although these patients consulted our clinic because their motor symptoms had progressed after spinal surgery, we found that there were no differences in the rate of disease progression and survival between two groups ($P > 0.05$).

Conclusions: Our results indicate that nearly half of all ALS patients have their disease complicated by cervical spondylosis, some of whom even underwent spinal surgery. Although the surgery did not obviously hasten the rate of disease progression and shorten survival, it lengthens the time from disease onset to diagnosis. A careful differential diagnosis for ALS is necessary before making decisions about spinal surgery.

P200 A POSSIBLE PROGNOSTIC ROLE OF ELECTROMYOGRAPHY IN ALS

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Keywords: prognosis, EMG, survival

Background: Electromyography (EMG) is important in ALS diagnosis because it can extend clinical findings by revealing lower motor neuron involvement in muscles otherwise considered as unaffected. EMG supports an ALS diagnosis if signs of active denervation and chronic partial reinnervation are found in at least two muscles in the cervical and lumbosacral spinal cord regions and in one muscle in the brainstem and thoracic spinal cord regions. If the role of neurophysiology in ALS diagnosis is well established and routinely applied, electrophysiological studies aimed to detect ALS prognosis are lacking.

Objectives: To investigate a possible prognostic role of EMG in ALS.

Methods: From 2000 through 2008, ALS patients diagnosed in our department underwent a standardized diagnostic neurophysiological protocol including: a) conduction studies (at least 4 motor nerves, and 3 sensory nerves): motor and sensory conduction velocity (CV), F-wave latency, distal composite motor action potential (CMAP) latency, duration and amplitude, and sensor nerve action potential (SNAP) amplitudes; b) needle examination (at least 6 muscles): spontaneous activity (fibrillation potentials, positive sharp waves, fasciculations), pattern of maximal contraction, Motor Unit Potentials (MUP) analysis. Survival curves of different clinical and electromyographical ALS subgroups have been compared to identify early predictors of ALS outcome.

Results: 103 patients (mean age at onset: 63 years; mean survival time: 39 months) underwent diagnostic EMG on average 15.5 months after onset. Fifty patients had upper limb onset (49%), 28 lower limb (27%), and 25 bulbar (24%) onset. Conduction studies parameters did not affect survival. On the contrary, survival was influenced by the presence/absence of fasciculations in the bulbar region ($p = 0.04$ Log-Rank Test), but not by the presence/absence of fasciculations in the cervical or lumbosacral regions. Fibrillations, positive sharp waves, or MUP features did not affect survival. A neurogenic pattern of maximal contraction in the bulbar, but not in the cervical or lumbosacral region, was associated with a worse prognosis ($p < 0.01$).

Discussion and Conclusions: Electrophysiological data can provide insights into the extent of motor unit loss due to the disease and they may have a prognostic role in ALS. The present study suggests that a neurogenic pattern of maximal contraction in the bulbar region is associated with a worse prognosis, probably because it is a marker of severe bulbar involvement. Moreover, the presence of fasciculations was associated with a worse disease course. Assuming that in ALS fasciculations arise from the motor unit, they can represent a sign of motor unit damage. Recently it has been suggested to include these potentials in ALS electrophysiological diagnostic criteria (1). These results underline

the importance of fasciculation potentials in ALS and suggest a prognostic role of this electrophysiological parameter.

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P201 INFLUENCE OF SYMPATHETIC PREDOMINANCE ON OUTCOME IN ALS PATIENTS

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Keywords: sympathetic hyperactivity, mortality

Background: ALS is a fatal degenerative nervous system disease caused by death of motor neurons in the motor cortex, brainstem and the spinal cord. Evidence is increasing to show that the autonomic nervous system is also affected in this disease and that patients exhibit impairment of parasympathetic function with sympathetic predominance (1, 2). Sympathetic hyperactivity and reduced heart rate variability have been associated with poor outcome in patients after acute myocardial infarction and with other cardiac diseases (1).

Objectives: To assess the role of increased sympathetic tone on mortality in ALS patients.

Methods: Fifty five patients with definite ALS according to El Escorial criteria (2000) (28 women and 27 men; average age 56.0 ± 10.3) comprised the study group. Patients with previous history of cardiac disease, impaired respiratory function and ALSFRS-R score below 35 were excluded from the study. A ten minute real time beat-to-beat ECG signal monitoring with heart rate variability analysis at rest was carried out in all patients at the beginning of the study. Spectral power of the low frequency component expressed in normalized units (LFnu-RRI) over 55 indicated sympathetic predominance. Clinical end-points included the beginning of non-invasive ventilation (NIV), placement of percutaneous endoscopic gastrostomy (PEG) and expected or unexpected (sudden) death. The follow-up period was 38 months. Statistical analysis was carried out using the Kaplan-Meier method. Differences between groups were measured by log-rank tests.

Results: At the end of the follow-up period, NIV was applied in 13 (23.6%) and PEG in 14 (25.5%) patients; 21 patient (38.2%) died, eight of whom died unexpectedly. Kaplan-Meier non parametric analysis and log-rank test showed no differences between patients with sympathetic predominance and patients without sympathetic predominance in relation to NIV and PEG application. In contrast, the overall mortality risk was significantly higher in patients with sympathetic predominance at rest ($p < 0.05$). The risk of unexpected death was also significantly higher in patients with sympathetic predominance at rest ($p < 0.05$).

Conclusions: The results of this study showed that sympathetic hyperactivity is a significant mortality risk factor in patients with ALS, regardless of the degree of respiratory insufficiency and dysphagia. Sympathetic predominance in association with nocturnal oxygen desaturation, which is known to occur in ALS patients, may significantly contribute to unexpected death. Tests of autonomic function may help to identify ALS patients at risk of sudden death.

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P202 VISUAL AND PROPRIOCEPTIVE POSTURAL CONTROL IN PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: posturegraphy, balance, sensory integration

Background: Falls are common in ALS and may occur before weakness. We previously employed the modified Clinical Test of Sensory Integration and Balance (mCTSIB) and reported postural orientation impairment as ALS patients nearly fell when they stood on foam with eyes closed, which indicates increased reliance on vision to maintain balance and equilibrium. We hypothesized that a lack of congruence between a subject's visual and floor environments results in (a) amplification of body sway, and (b) an inability to adapt when sensory cues are either unreliable or completely absent.

Objectives: To investigate the adaptability to altered and incongruent visual and proprioceptive feedback situations during balance control in people with recently diagnosed ALS.

Methods: Seven subjects (mean (M) age = 63.5 years) with early stage ALS were evaluated using the Equitest Sensory Organization Test (SOT) protocol. A forceplate measured anterior to posterior center of gravity displacements in 6 sensory conditions: (a) 3 Fixed Support (FS) Surface conditions with eyes open (C1), eyes closed (C2), or eyes open/Sway-Referenced (S-R) surround (C3); (b) 3 S-R Support Surface conditions with eyes open (C4), eyes closed (C5), and eyes open/S-R surround (C6). Six equilibrium (ES) scores (C1-C6), one for each condition, were generated to identify sensory organization abnormality. Additionally, six movement strategy scores (MS1-6) measured horizontal shear forces during each of the six successive SOT conditions. The relative use of movement about the ankle and hips and upper body to maintain balance during the SOT was reflected in the MS scores. MS scores around 100 reflected a predominant use of ankle strategies to maintain balance, whereas low scores are indicative of the use of hip strategies to restore stability.

Results: Twenty-two of 63 (34.9%) trials on C4-6 resulted in equilibrium scores that fell below norms of healthy age matched adults. There were 9 falls among 4 subjects during C5 and C6. A 2X3 ANOVA (Floor by Vision) (C1-C6) produced a significant floor by vision interaction

($F(2,12) = 10.37$, $p < 0.01$). Subjects scored significantly better in all FS surface conditions (C1-3) than for S-R support conditions (C4-6), creating the interaction. Also, the FS conditions ($F(1,6) = 360.58$, $p < 0.001$) produced a significantly higher MS score ($M = 94.75$, $SD = 5.41$) than the S-R Support conditions ($M = 15.94$, $SD = 19.66$).

Discussion and Conclusion: These results suggest that when visual and proprioceptive cues are unreliable or absent, the remaining sensory messages become unintelligible or unusable, thus supporting the stated hypotheses. Therefore, it is important to use rehabilitative techniques which expose people with early ALS to various conditions which remove visual and proprioceptive input or make sensory inputs incongruent.

P203 PROSODIC STUDY OF THE SLOW SPEAKING RATE IN MOTOR NEURON DISEASES

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Keywords: PLS, dysarthria, prosody

Background and Objectives: The purpose of this study is to investigate the slow speaking rate in dysarthria associated with Primary Lateral Sclerosis (PLS) that leads to a "spasticity" of the speech organs. This study is focused on the consequences of the slow speaking rate on prosodic aspects of the patients' production and the implications of the temporal disorders from a perceptual point of view in term of "fluency".

Methods: The data for this study were collected from 40 French subjects including 20 patients diagnosed with PLS and 20 gender-matched controls. Subjects were instructed to read a short sentence organized in 4 breath groups and 21 syllables (24 consonants and 21 vowels). The temporal and intonation patterns of the 40 sentences were then synthesized with Mbrola in order to control for variation in the productions other than F0 and duration. Eight expert listeners were instructed to judge the synthesized stimuli in terms of 'fluency' on a 4 step scale.

Results: Dysarthric speakers in this study did modulate prosodic parameters (F0 and duration) but to a smaller extent than the control speakers, leading to the typical monotonous speech in spastic dysarthria.From a segmental point of view, the reduced speaking rate observed in the dysarthric speakers was observed in the duration of all segments but was more manifested for vowels than for consonants, particularly for the nasal and back rounded vowels. From a perceptual point of view, the fluency of the dysarthric speakers was judged to be worse when the duration of the vowels and that of the unaccented syllables was inconsistent with the pattern observed for the controls.

Conclusions: This study allowed us to better analyse the effects of an upper motor neuron lesion on the acoustic parameters of speech. We expect that our findings in PLS patients will allow us to better discriminate the upper motor neuron from the lower motor neuron involvement in the dysarthria of patients with ALS.

P204 INFLUENCE OF THE AGE OF ONSET IN THE ALS PROGNOSIS

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Keywords: age of onset, prognosis, initial form

Background: Previous studies suggest that late age of onset and bulbar onset can determine a worse prognosis in ALS patients.

Objective: To evaluate the influence of the age of onset and the form of the disease at onset on the ALS patient's outcome.

Methods: A descriptive study of consecutive patients who attended the ALS Unit between 2006 and 2009 was performed. The introduction of non-invasive ventilation (NIV), the placement of gastrostomy, the use of wheelchair and death were the parameters studied.

Results: Seventy-seven patients (43 males; 55.8%) were analysed, median age of onset of 59.45 years. Age of onset groups: 11 developed symptoms at less than 45 years (14.3%), 17 at more than 70 years (22.1%) and 49 between 45 and 70 years (63.6%). Average time to diagnosis was 21.71 months. Sixty-eight patients (88.3%) were treated with riluzole and 5 with lithium (6.5%). NIV was introduced in 28 cases (36.4%), gastrostomy in 20 (26%), wheelchair in 40 (51.9%) and 30 patients died (39%) with an average of survival of 43.77 months (range: 4–192 months). There is a negative correlation between the age of onset and the delay in the diagnosis (-0.352, $p < 0.05$), the time of evolution to needing an NIV system (-0.686, $p < 0.05$), to needing a gastrostomy (-0.607, $p < 0.05$) and the time of survival (-0.611, $p < 0.05$). In the group with disease onset after 70 years of age a statistically significant association was observed with NIV use (58% vs. 34.7% vs. 9.1%; $p < 0.05$). No significant data were found in relation to the initial form of the disease. The frequency of bulbar onset in the group with onset at less than 45 years is less than in other groups of age (1 case, 9.1%).

Conclusions: A later age of onset of the symptoms involves a more rapid diagnosis of the disease, an earlier need for NIV and gastrostomy and an early death. Bulbar onset is less frequent in younger patients. The initial form of the disease does not determine the ALS outcome.

P205 AMYOTROPHIC LATERAL SCLEROSIS IN YOUNG-ADULT PATIENTS

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Keywords: young, survival, signs

Background: Amyotrophic lateral sclerosis is clinically and biologically a heterogenous disorder. The sporadic form of ALS (sALS) affects people of all ages, however the mean age of onset varies between 55–65 years with the median age of

onset 64 years. Only around 10% of cases have an onset before the age of 40 years. Juvenile sporadic onset ALS is also being recognized. The course of disease of young-adult onset sALS and juvenile sALS cases can differ as compared to sALS with typical age of onset.

Objectives: To investigate the clinical differences between young-adult sALS cases (yoALS; onset before age 40 years) and patients with typical onset of sALS (ALS; adult-onset type).

Methods: Based on the clinical database of the MND Center in Krakow we retrospectively analyzed clinical features and course of the disease of 481 sALS patients, diagnosed according to El Escorial criteria. We found 45 yoALS patients and compared them with 436 patients affected by adult-onset ALS.

Results: The ratio of men to women was 1.6:1 in yoALS cases and 1:1 in the ALS group. We found that the majority of yoALS patients demonstrate the limb onset form of the disease – 87% as compared to remaining ALS – 31% ($p < 0.01$). First signs were located frequently in upper limbs in yoALS – 44% (ALS – 31%). In yoALS we observed predominant upper motor neuron involvement in 58% whereas in ALS this was 43%. All patients showed a clearly progressive course of disease, however yoALS had longer median survival: 39 months as compared to 29 months in ALS ($p < 0.05$).

Discussion and Conclusion: There are few data reporting the different course of ALS in young-adult patients. Male predominance, longer survival, more symmetrical clinical presentation with predominance of upper motor neuron signs have been demonstrated in young ALS patients. In the presented yoALS series we also noticed significantly longer survival and a quite distinct clinical picture, however without male predominance and symmetry of signs. Our observation might be genetically based and regional.

P206 RATE OF DECLINE AS PROGNOSTIC INDICATOR IN MOTOR NEURONE DISEASE

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Keywords: rate of decline, markers of disease progression, survival

Background: Motor neurone disease (MND) is a neurodegenerative disease, ideally managed in a multidisciplinary care setting. In terms of optimizing therapies in MND, there remains a clear need to develop prognostic markers.

Objectives: Consequently, the present study aimed to develop a simple measure to reflect the rate of disease progression and thereby quantitatively predict MND survival times.

Methods: A prospective study was conducted involving 92 patients recruited from a multidisciplinary motor neurone disease clinical service. Baseline characteristics (age, gender, weight, disease duration, family history, amyotrophic lateral sclerosis functional rate scores (ALS FRS)), and respiratory data were each recorded and dynamic measures were continued throughout the period of clinical follow-up.

Results: A novel rate of decline (ROD) value was developed from sequential measures of ALS FRS, spirometry and disease duration data. All ROD values were then combined into a representative cohort plot for the calculation of survival times. ALS FRS ROD values demonstrated significant inverse correlations with disease progression; the shorter the disease progression the higher the ROD value. Survival analysis using Kaplein Meier curves established significant associations between ROD based on ALS FRS scores, spirometry and mean survival times (MST). Those with higher ROD values had much lower mean survival times compared to patients with lower RODs. For each significant ROD parameter (ALS FRS total, bulbar ROD, respiratory ROD, FEV1 ROD and FVC ROD) representative plots were generated.

Discussion and Conclusions: Rate of decline values based on ALS FRS and spirometry are sensitive markers of disease progression and quantitative predictors of survival. These values were also useful predictors of individual survival times, highlighting the potential of ROD as a clinical assessment tool in monitoring progression, assisting in the appropriate timing of treatments and evaluating effectiveness of treatment in slowing disease progression and thereby potentially improving survival.

P207 EFFECTS OF NIPPV ON THE PROGNOSIS OF ALS PATIENTS WITH RESPIRATORY ONSET

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Keywords: respiratory onset, NIPPV, prognosis

Background: Respiratory muscle involvement is a major prognostic factor in ALS patients. Most ALS deaths are due to respiratory failure. It is now proven that NIPPV improves the prognosis of ALS patients with respiratory insufficiency. Respiratory onset is a presenting symptom of ALS in a small number of patients. Multiple case reports in the literature described motor neuron disease presenting with respiratory failure but it can start more insidiously and can be diagnosed before emergency intubation is required. Effect of NIPPV on the prognosis of ALS with respiratory onset has been assessed only in a few studies.

Objectives: To review the clinical characteristics and prognosis of ALS patients with respiratory onset and evaluate the effect of NIPPV on the prognosis of these patients.

Methods: We performed a retrospective analysis of all cases reviewed at the ALS Centre of Marseille from 2003 to June 2008, entered in a database. All included patients met the criteria for definite or probable ALS (revised El Escorial criteria). The database was searched for entries with respiratory onset or NIPPV use within 13 months after the onset of disease. Respiratory onset was defined if the first symptom of muscular weakness was effort dyspnea, dyspnea at rest or orthopnea.

Results: Seventeen ALS patients had a clear respiratory onset; they were all men. The mean age at symptom onset was 65.5 (13) years and mean time to diagnosis was 7 (3.6)

months. Only 4 patients were alive at the time of the analysis. One patient required emergency intubation and died 2 months after tracheostomy. Camptocormia or dropped head were observed in 8 patients in the 3 months after diagnosis. All patients were ambulatory at the first evaluation and 12 of 13 patients stayed ambulatory at the last evaluation before death. NIPPV was offered early in 16 patients (median 8 months after onset of disease) and was well tolerated in 14 of them. The mean survival time to death or permanent assisted ventilation in patients with NIPPV was 15 (5.6) months. Loss of weight before diagnosis was important in 16/17 patients (mean = −4.7 BMI points) and most of them (8/13) stabilised their weight after NIPPV.

Conclusions: ALS patients with respiratory onset still have a poor prognosis. NIPPV initiation has to be offered early because it improves symptoms, general state of health and survival time. The loss of weight can be slowed down with NIPPV. Autonomy is conserved practically until death for almost all the patients. Certain clinical symptoms appeared to be more frequent: camptocormia, widespread fasciculations and a severe initial loss of weight.

P208 NATURAL HISTORY OF UPPER MOTOR NEURON-DOMINANT ALS

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Keywords: upper motor neuron, prognosis, UMN-dominant ALS

Background: Among prognostic factors in ALS, clinical features with a predominance of UMN involvement have been repeatedly reported to predict a more benign course. A new ALS category named "UMN-dominant ALS" and defined as "due predominantly to UMN signs but with minor electromyogram (EMG) denervation or LMN signs on examination" has been proposed (1).

Objectives and Methods: To describe clinical and laboratory features of 20 patients with UMN-dominant ALS. To longitudinally analyze their disease course according to disability progression and further define their relationship with ALS comparing disability progression and outcome data with those of a sex-, age at onset-, and disability-matched group of typical ALS patients.

Results: Ten women and 10 men with a diagnosis of UMN-dominant ALS according to Gordon's criteria (1) were evaluated. Their mean age at onset was 58.6 years (range, 48–61; median = 59.5). The onset was in the spinal region (lower limbs) in 12 patients and in the bulbar region in the remaining 8 patients. At the last evaluation, after a mean disease duration of 7.7 years (range, 3.6–14.6, median = 7) and a mean follow up at our clinic of 3.4 years (range, 2–10, median = 2.7), all patients but one had progressed to a tetrapyramidal syndrome with pseudobulbar features of varying degrees. Muscle atrophy was observed in 13 patients. In 12 of them it was localized to intrinsic muscles of one or both hands. Although all patients complained transiently of fasciculations, we observed fasciculations in only five patients on examination, mainly in the distal muscles of upper limbs and quadriceps femoris. No patient complained of respiratory problems and their mean respiratory ALSFRSr score was 11.4/12 (range, 10–12; median = 12). When comparing disease progression in UMN-dominant ALS patients with ALS controls, by evaluating ALSFRSr score and FVC value changes in the two groups of patients, we could observe a distinct outcome between the two groups as early as 6 months of follow up. At the time of this study, all UMN-dominant ALS patients were alive with a mean disease duration of about 8 years, whereas 16/20 ALS controls had died or tracheostomized, their median survival being as long as 41 months.

Discussion and Conclusions: These findings suggest both a different pattern of disability and a longer survival in UMN-dominant ALS patients compared to classical ALS. The most outstanding result is the persistence of a normal respiratory function over time which mainly underlies the good prognosis in these patients.

Reference:

1. Gordon PH, Cheng B, Katz IB *et al.* Neurology 2006;66:647–653

P209 TREATABLE FLAIL-LEG SYNDROME

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Keywords: flail-leg syndrome, intravenous immunoglobulin, treatment

Background: Flail-leg syndrome, akin to flail-arm syndrome, is a motor neuron disease characterized by flaccid lower limb (LL) paresis, generally asymmetric. It typically has a slower progression and derives from degenerative lower motor neuron loss in the lumbar segments. We present 2 patients who clearly improved on intravenous immunoglobulin (IvIg).

Cases: Case 1 is a 41-year-old female who presented with progressive asymmetric LL paresis for over 5 years. At first the weakness was fluctuating with periods of improvement followed by partial improvement, before a gradual weakness was established. On observation she had bilateral LL weakness and atrophy, more severe on the right distally (foot steppage). She was wheel-chair confined. Deep tendon reflexes (DTR) were absent and mild sensory changes were present distally. Muscle strength and DTR were normal in the upper limbs (UL) and bulbar region. Case 2 is a 63-year-old male with progressive LL weakness for 2 years. He referred a moderate discomfort in the left L5 root territory, but no other sensory symptom. On observation, LL weakness and atrophy was bilateral but more severe on the left side, distally, with foot steppage. DTR were very weak in LL, but normal elsewhere. Strength was normal in UL and bulbar region. Sensory examination disclosed mild changes on the left L5 dermatome. The patient needed a cane to walk.
In both patients blood tests were normal (including immunoelectrophoresis) and CSF analysis disclosed mild increase of the protein content (50 mg/100 ml) with oligoclonal IgG bands in the first patient. Anti-ganglioside antibodies were negative. Detailed MRI spinal cord investigation was normal. Neurophysiological investigation showed normal motor conduction velocities, normal CMAP amplitudes and sensory potentials, but F-waves were absent in case 1. Needle sampling showed no active denervation in case 1, but large motor units and active denervation were observed in case 2. Lumbar roots and brain magnetic stimulation suggested proximal LL nerve conduction block in case 1. On

IvIg treatment the LL muscle power clearly improved in both patients and this amelioration persisted over time. Introduction of steroids was tried twice in case 1, but it clearly increased weakness in both occasions. Today, both patients are able to walk without any support on IvIg only.

Discussion: This report indicates that some patients with progressive LL weakness suggesting flail-leg syndrome are affected by an immunological-associated condition. Normal conventional nerve conduction studies do not exclude proximal nerve lesion in LL and a trial of IvIg treatment is valid in atypical cases.

P210 THE NATURAL HISTORY OF LEG ONSET ALS

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Keywords: leg onset, prognostic model, survival

Background: Progression of ALS is variable, although the pathophysiological substrate for this remains elusive. Patients benefit from prompt, accurate prognostic information to optimise decision-making and care planning. The symmetrical, slowly-progressive upper-limb ALS phenotype of 'flail arm' is recognised early, but the entity of 'flail leg' is subject to post hoc definition, and its early distinction from slowly-progressive lower limb onset ALS less clear.

Methods: A tertiary clinic-based observational study was performed. Clinical information was prospectively collected from ALS patients with lower limb symptom onset from 2003–8. New weakness or wasting was taken as symptomatic spread to another limb. Use of riluzole was recorded. Kaplan-Meier survival analyses and multivariate Cox proportional hazards modelling were used to explore significant prognostic factors.

Results: Seventy-five lower limb onset ALS cases were identified (52% male). Mean age of onset was 60 years (range 35–84). The proportion of patients alive at censoring was 32%. The mean latency from symptom onset to referral was 27 months (SD 29, range 2–216). Median survival of lower limb onset ALS cases by Kaplan-Meier analysis was 54 months (95% CI 38–69). Mean time to symptom progression was 23 months (SD 31, range 1–210). The site of next involvement was the contralateral lower limb in 69% and ipsilateral upper limb in 31%. There were no cases of consecutive involvement of the contralateral upper limb or bulbar region. 42% of patients reported riluzole use. Significant prognostic variables in univariate Kaplan-Meier analysis included: referral latency and time to progression ($p < 0.0005$). There was no significant difference in survival between those whose disease spread next to the contralateral lower limb versus ipsilateral upper limb (median survival 52 versus 39 months; $p = 0.5$). The use of riluzole was associated with an apparently adverse survival (riluzole use median survival 38 months, no riluzole use 57 months; $p < 0.02$), but of the six factors studied, those independently affecting survival within the Cox proportional hazards model were time to progression and referral latency only ($p < 0.0005$). A regression function was fitted for survival plotted against time to progression in those patients who had died. Overall survival in months from symptom onset was approximately two years plus the time to progression in months ($r2 = 0.51$).

Conclusions: Lower limb onset ALS can be a slowly progressive condition whether there is spread to the contralateral limb, or spread to the ipsilateral arm. The limitation in site of consecutive involvement clinically supports previous work on focality and spread of pathology. The time taken for progression of symptoms beyond the lower limb of initial onset in sporadic ALS is a major prognostic factor and can be used to estimate overall survival. We suspect that patients with a more slowly progressive disease are less inclined to take riluzole.

P211 ANALYSIS OF SLEEP CHARACTERISTICS IN POST-POLIO SYNDROME PATIENTS

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Keywords: post-polio syndrome, sleep, polysomnography

Introduction: The main MND/Post-Polio Syndrome symptoms are new weakness, new atrophy, fatigue, pain and sleep disturbances. The polysomnographic study is the gold standard to analyze sleep disturbances. Some authors believe there is an impairment of the bulbar region from an anatomical and pathological viewpoint, and, for this reason, an impairment of the center responsible for sleep can occur more frequently than is mentioned. These findings can have a direct relationship to the clinical manifestations of PPS. Our experience led us to hypothesize that PPS patients with sleep complaints present sleep disturbance and physical symptoms. We correlated sleep disturbance to the clinical complaints of PPS patients.

Methods: Sixty patients (39 female) with PPS, mean age 46.8 ± 11.3 years, underwent a personal interview and answered the scales: 1) Function scale (Barthel index); 2) Pain analogical scale; 3) Fatigue severity scale (FSS); 4) Epworth sleepiness scale (ESE); 5) Stanford Sleepiness scale (SSS); 6) Mini-Sleep Questionnaire (MSQ). In addition, they completed a polysomnographic exam at the Sleep Institute. The scales and the polysomnographic results were correlated using the Spearman correlation test ($p < 0.05$).

Results: The sleep quality of PPS patients is decreased (total sleep time, minutes $= 324.9 \pm 73.3$; sleep efficiency, $\% = 69.2 \pm 14.9$; arousal index, number per hour (n/h) $= 9.9 \pm 11.5$). They present more apnea and hypopneas (AHI, n/h $= 6.2 \pm 14.5$) and periodic limb movements (PLM, n $= 45.7 \pm 105.3$) than the overall population. However the oxygen saturation and the carbon dioxide level did not change. When comparing the polysomnographic variables with fatigue, pain, daytime hypersomnolence and function, people with a higher fatigue level do not change stages many times during a night; pain is not related to SpO_2 or apnea index, but with central and mixed apneas. People with a higher daily sleepiness wake more during the night, present higher PLM index, and also present a higher pressure of carbonic gas ($P_{ET}CO_2$).

Conclusions: Sleep quality is decreased but SpO_2 and $P_{ET}CO_2$ are not changed in PPS patients. There are few positive correlations between polysomnographic findings and PPS symptoms. These findings led us to hypothesize that sleep quality of PPS patients is decreased compared to the overall population of the same age.

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THEME 9 COGNITIVE AND PSYHOCOLOGICAL ASSESSMENT AND SUPPORT

P212 FRONTOTEMPORAL DEMENTIA IN 2 FAMILIES WITH TARDBP A382T MUTATION

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Keywords: frontotemporal dementia, positron emission tomography, TARDBP

Background: TAR DNA-binding protein 43 (TDP-43), encoded by the TARDBP gene, has been identified as the major pathological protein of frontotemporal dementia (FTD) with ubiquitin-immunoreactive inclusions (FTLD-U) with or without amyotrophic lateral sclerosis (ALS) and sporadic ALS. Subsequently, mutations in the TARDBP gene have been detected in 1% of ALS patients (both familial (FALS) and apparently sporadic (SALS) cases), suggesting that such mutations may be linked to ALS pathogenesis. However, there is only one description of two ALS patients with TARDBP gene mutations and FTD.

Objectives: To describe cognitive abnormalities in two Italian FALS cases with TARDBP gene mutations.

Methods: A total of 7 index cases with different TARDBP mutations have been assessed with a neuropsychological battery evaluating executive functions, memory/learning, attention/concentration, language and visual-spatial functions. The patients underwent ^{18}F-FDG-PET/CT scans. The scans were acquired by the Discovery STE PET/CT System (General Electrics), combining a helical multi-slice CT scanner and a designed BGO block detector PET tomograph, in 3D modality.

Results: Two out of the 7 index cases with a TARDBP mutation showed a FTD. Both cases carried the A382T mutation. The first patient, a 43 year-old male, developed dysarthria at the age of 42, followed by hand wasting. At the neuropsychological examination he showed impulsivity, distraibility and semantic impairment. The diagnosis was predominantly behavioural FTD in bulbar ALS. The PET/TC showed a hypometabolism in frontal areas (associative and mesial areas) and in the right temporal associative areas. His mother, carrying the same TARDBP mutation, developed upper limb ALS at the age of 69 and is still alive after 12 months. The second patient, a 61 year-old woman, developed dysphagia and dysarthria when 59. At the time of our observation, she showed an apathetic behaviour, with a dysexecutive syndrome. The PET/CT showed a severe predominantly frontal hypometabolism. The patient's father, who developed bulbar ALS at 78, had shown similar neuropsychological disturbances for two years before the onset of ALS.

Conclusions: We have described two apparently unrelated FALS cases carrying the A382T TARDBP mutation, who showed a FTD that had been confirmed with a neuropsychological assessment and a PET/TC scan. The FTD was also present in the affected relatives of the first patient, indicating a co-segregation of ALS and FTD in this family. These findings indicate that FTD may develop in patients with ALS carrying TARDBP mutations.

P213 THE VOLUME OF AMYGDALA DID NOT CORRELATE TO PAIN IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: Amyotrophic lacteral sclerosis (ALS) is a fatal neurodegenerative disease. In the late stages, pain is one of the most common symptoms of some ALS patients. The amygdala is associated with the negative emotions of pain. Recently, some studies indicated damage to the amygdala in ALS. This study explored the possibility of a relationship between amygdala damage and pain in ALS patients.

Methods: Twenty-eight definite or probable ALS patients without dementia and 30 matched controls were included. Amygdala volumes were measured by IBASPM in MRI. ALS-FRS, Short-Form McGill Pain Questionnaire, Hamilton Anxiety Scale and Hamilton Depression Scale were collected.

Results: The comparison of amygdala volumes between the patients and the controls showed no significant difference. The comparison between patients with pain and patients without pain also showed no significant difference. The amygdala volumes and SF-MPQ emotion scores were uncorrelated. ALS-FRS was higher in patients with pain than patients without pain ($p < 0.05$). SF-MPQ scores and ALS-FRS were negatively correlated. ALS-FRS and HAMA scores were negatively correlated. SF-MPQ scores and HA-MA\HDMA scores were positively correlated.

Conclusions: No significant alteration of the amygdala volume in ALS patients was found and no relationship between amygdala damage and pain was found. In those with more disabilities, more pain occurred and to a greater degree; meanwhile anxiety was more severe. The pain of ALS patients might be influenced by anxiety and depression.

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P214 FEATURES AND RELATED FACTORS OF COGNITIVE FUNCTION IMPAIRMENT IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: event-related potentials, neuropsychological test

Background: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease involving both upper and lower motor neurons selectively. Evidence from neuroimaging studies, neuropsychology, neurophysiology and neuropathology suggest impairment of structure and function outside of the motor system. Some ALS patients experience changed cognitive function during the course of the disease.

Objectives: To observe the changes of event-related evoked potentials (P300) in ALS patients and to investigate the features and related factors of cognitive dysfunction. The relationship of the components of P300 and neuropsychological tests were also analyzed.

Methods: P300 and comprehensive cognitive function tests including attention, memory, executive function, visual spatial skills and language were investigated and analyzed in 30 patients with the diagnosis of sporadic ALS and 30 normal controls.

Results: In ALS patients, the latencies of N2, P3 and N2P3 were significantly increased compared with the control group while no differences were found in the latencies of N1 and P2 as well as the amplitudes of the waves mentioned above. In the attention test, the score of ALS patients was lower than controls. Memory testing showed decreased scores in AVMT1-AVMT3 and AVMT4 but no difference in AVMT5. The most obvious changes in executive tests were perseverance and resistance to interference. No significant difference was found between the two groups in the visuospatial processing test but in the language test the ALS patients had poor performance. As to the relationship between components of P300 and cognitive function in ALS patients, age had a negative correlation with the latencies of P3 and N2P3 and the course of the disease correlated positively with the latency of N1. No correlation was found between P300 and occupation as well as education. ALSSS of limb function in ALS patients had negative correlation with P2 and N2P3.

Conclusion: These findings showed the presence of cognitive deficits in sporadic ALS patients. Among the components of P300, latencies of N2, P3 and N2P3 were prolonged significantly in ALS patients, which electrophysiologically suggested cognitive impairment. Cognitive functions such as attention, memory, executive function and language were also changed. Age, course of the disease and ALS functional score were among the factors that influenced the cognitive impairment in ALS patients.

P215 HEMISPHERIC LATERALIZATION OF EMOTIONAL PROCESSING IN ALS

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Keywords: fMRI, emotional processing, lateralization

Background: Emotional processing may be altered in ALS and previous studies have revealed evidence for reduced arousal for negative stimuli.

Objectives: To explore anatomic functional correlation in processing of aversive information in ALS patients with normal brain MRI.

Methods: We examined the performance of 9 non-demented ALS patients and 10 healthy controls on two fMRI tasks, consisting of an emotional valence attribution task and a recognition memory task of unpleasant versus neutral words. Alternating blocks of unpleasant and neutral trials were presented. During the emotional decision task, subjects were asked to select one of three unpleasant or three neutral words. During the memory task, subjects were asked to recognize those words, both unpleasent and neutral, that were presented during the emotional decision task. fMRI images were detrended, filtered and co-registered to standard brain coordinates.

Results: In comparison to controls ALS patients showed increased brain activation in the left hemisphere, and reduced activation in the right hemisphere in both tasks. Control subjects showed a more elevated brain activation in the right middle frontal gyrus during the emotional decision task and in the right posterior cingulate during the memory recognition task compared with ALS patients.

Discussion and Conclusions: These results may be related to an altered sensitivity to negative emotional cues further confirming the abnormal emotional processing in ALS.

P216 PSEUDOBULBAR AFFECT: BETTER UNDERSTANDING THROUGH RESEARCH ON A SOCIAL NETWORK

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Keywords: pseudobulbar affect, emotional lability, internet

Background: Pseudobulbar affect (PBA) is characterized by exaggerated/involuntary emotional outbursts and occurs in patients with degenerative neurological conditions such as ALS and MS. There are currently no FDA approved treatments for this disorder, which has been proven to

negatively impact patients' quality of life. In a recent trial, approximately 300 patients were needed to study the safety and efficacy of the combination of Dextromethorphan and Quinidine (Zenvia™) for the treatment of PBA. In a novel use of online social networking, some patients with ALS were recruited for the trial using the website PatientsLikeMe. Following the close of recruitment, a survey was sent out to ALS patients to learn more about their experiences of PBA.

Methods: Following Avanir's recruitment for the Zenvia™/ STAR trial through the PatientsLikeMe Clinical Trial Access program, a survey was developed in partnership between the two organizations to find out more about the experiences of ALS patients. The survey was sent out to 2,851 ALS patient members, all of who had joined PatientsLikeMe prior to the end of the clinical trial.

Results: Within a week of the survey invitation going out, responses were received from 341 ALS patients (12% of those invited). 84 patients (3%) opted out of the survey and 18 patients (1%) started the survey but did not complete it. The denominator may include some deceased patients, so the actual response rate from eligible participants is likely to be higher. Despite being a widely recognized symptom of ALS, 20% of patients were unaware that PBA was associated with their condition. Patients were most familiar with the terms "Emotional lability" (63%), "Uncontrollable emotions" (41%), and Pseudobulbar Affect / PBA (38%). Two thirds of patients (66%) reported some kind of recent experience of an uncontrolled emotion, but only 51% of patients reported these to their physicians. Patients experiencing labile laughing & anger in combination were more likely to have reported these episodes to a clinician (78%), compared to crying & anger in combination (35%). Among survey respondents, 12 ALS patients (4%) identified themselves as having taken part in the STAR trial, all via their physician or specialist ALS care center.

Conclusions: PBA is a common problem in ALS, but a fifth of patients were unaware that there is a link between the two. This is congruent with previous studies reporting physicians are less likely to warn patients about psychological and cognitive symptoms than physical ones. Only about half of patients with PBA actually report it to their physician and it may only be the most incongruent episodes that are recognized as pathological. Social networking should be further evaluated as a tool for informing patients about current research alternatives.

P217 STUDY OF EMOTIONAL REACTIVITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: memory, anxiety

Background: Seldom described as depressed, ALS patients seem to cope efficiently with their diagnosis. It thus appears important to identify the elements at the origin of this state.

Objectives: To show the existence of a fundamental emotional dysfunction in ALS and to answer whether positive emotions are perceived more intensely and whether positive information is memorized better.

Methods: Ten ALS patients, 7 men and 3 women (62.7 years ± 9.6), with mean disease duration of 28 months (± 13) and an average ALSFRS score of 36.6, and 10 control subjects, 7 men and 3 women (62.5 years ± 10.3) were evaluated with an anxiety scale (STAI), a depression scale (BDI-II), a normalized corpus of "emotional words" of valence and intensity variables and a battery of executive tests.

Results: ALS patients pointed out more positive words than negative ones from the normalized corpus of "emotional words", with the two recalls (recall 1: $p = 0.0117$; recall 2: $p = 0.0051$). Only the negative words were significantly better recalled by the control group with recall 1 ($p = 0.0284$) and 2 ($p = 0.0022$). In addition, ALS patients did not have higher depressive intensity ($p = 0.7317$) compared with controls, but they had higher anxiety ($p = 0.0233$).

Discussion and Conclusions: The main results support the hypothesis that ALS patients memorize more positive information, this tendency being accentuated with time. With no correlation having been highlighted, it appears that these results cannot be related to a dementia, a major executive dysfunction, or an anxious and/or depressive symptomatology. It is therefore possible to consider that in ALS, an adaptive mechanism aimed at improving the capacity of adaptation and to maintaining a positive image is a characteristic of the patients.

P218 PSEUDOBULBAR AFFECT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS CORRELATES WITH IRRITABILITY AND AGITATION

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Keywords: FTD, pseudobulbar affect

Background: Pseudobulbar affect (PBA) is a neurologically-based affective disorder marked by involuntary and excessive displays of laughing or crying. PBA prevalence rates are highest among stroke, multiple sclerosis and amyotrophic lateral sclerosis (ALS) patients. The Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report measure, is a commonly used instrument to measure PBA. Little is known about associated behavioural and emotional features that accompany PBA.

Objective: To determine whether other forms of emotional dysregulation such as irritability, agitation, and euphoria are associated with PBA. To determine the relationship between depression and PBA. To determine whether ALS patients with PBA with a preponderance of crying have higher levels of irritability and agitation and whether those with a preponderance of laughing have higher rates of euphoria.

Methods: Patients ($N = 22$) were recruited consecutively from a multidisciplinary ALS Center and were requested to complete the CNS-LS, the Neuropsychiatric Inventory-Questionnaire version, the UCSF Pseudobulbar Affect Questionnaire, the Frontal Behavioral Inventory and the Beck Depression Inventory-II.

Results: As measured by a score of 2 or more on the NPI-Q on respective items, 24% of the sample were identified as having irritability. Agitation/aggression was present among 33% of the sample. The mean level of depression was in the mild range (BDI-II mean of 13.6 (7.0)), with 24% of the patients rating themselves as having moderate levels of depression and no patients having severe levels. A majority

of the patients had a preponderance of crying (52%), 24% had a preponderance of laughing, and 24% had equal amounts of each. A 2×3 ANOVA model revealed ($F = 6.28$; $p < 0.009$) that patients with a preponderance of laughing had higher levels of agitation/aggression, as compared with patients with a preponderance of crying, contrary to our hypotheses. No difference was found for the prevalence of euphoria. No statistical associations were present between PBA and level of depression, sex, education level, age, length of illness, illness severity, or breathing capacity.

Discussion and Conclusions: PBA is a disabling syndrome for many ALS patients yet is well treated with available medications. The PBA syndrome in ALS patients appears to be a distinct syndrome, separate from the depression seen in ALS patients. ALS patients' PBA syndrome may be better conceptualized as a broader spectrum of emotional disinhibition, including the presence of irritability and agitation. Patients with a preponderance of laughing may be more susceptible to this anger-dysregulation and patients with crying may be less vulnerable.

P219 COGNITIVE PROFILE IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS: A NEUROPSYCHOLOGICAL AND EVENT-RELATED POTENTIALS STUDY

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Keywords: event-related potentials, neuropsychological assessment, cognitive dysfunction

Background: Evidence of the presence of extra-motor involvement in patients with Amyotrophic Lateral Sclerosis (ALS) has been provided by recent neuropsychological, electrophysiological and functional brain imaging studies.

Objectives: To investigate the cognitive functions in non-demented ALS patients using neuropsychological assessment and auditory event-related potential (ERP) recordings and to verify the possible relationship among clinical, neuropsychological and ERP data.

Methods: Twenty-five ALS patients and 17 age and education-matched controls participated to the study. Dementia or major depression were excluded by clinical interview and by screening cognitive and affective domains using the Mini Mental State Examination and the Beck Depression Inventory, respectively. A neuropsychological evaluation assessed attention, language, memory, executive functions and intelligence. ERPs were evaluated with an active auditory odd-ball paradigm with standard (80%) and rare (20%) stimuli. Functional disability was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R).

Results: ALS patients performed significantly worse than controls in Semantic Verbal Fluency, Letter Verbal Fluency and Wisconsin Card Sorting Test (WCST). Regarding the late ERP components (N200 and P300), we did not find significant differences between ALS and control groups. A linear regression analysis showed a significant relationship between age and P300 latency and amplitude in control subjects but not in ALS patients. In ALS patients, correlation analyses showed a significant correlation between disease duration and neuropsychological tests (Letter Verbal Fluency, Trail Making Test-TMT A and B) and between ALSFRS-R and neuropsychological tests (Memory Prose and WCST). Moreover, the correlation analyses between clinical data and late ERP components revealed a significant relationship between disease duration and P300 amplitude. Finally, we found significant correlations between neuropsychological tests and P300 parameters: Raven's Coloured Progressive Matrices and P300 amplitude, Digit Span Forward and P300 latency, Digit Span Forward and P300 amplitude, Digit Span Backward and P300 latency, WCST and P300 amplitude, Memory Prose and P300 latency.

Discussion and Conclusions: Neuropsychological performances of ALS subjects suggest mild deficits of executive function, not attributable to motor impairment. Moreover, in ALS patients, the correlation analyses among clinical, neuropsychological and ERP data indicate that the progression of the disease affects cognitive performances and late ERP components (in terms of reduced amplitude and delayed latency) regardless of age and support the hypothesis that cognitive performance of ALS patients, when attentional, executive and working memory resources are requested, may be related to P300 latency and amplitude.
These findings, in agreement with the literature, confirm the presence of mild cognitive deficits in non-demented ALS patients, supporting the hypothesis of a pathological involvement beyond motor areas.

P220 VERBAL FLUENCY DEFICITS IN ALS: LANGUAGE OR EXECUTIVE DYSFUNCTION?

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Keywords: executive-functions, language, verbal fluency

Background: The most striking and consistently reported cognitive deficit in ALS is on tests of verbal fluency. This deficit has been shown to be independent of motor disability. Deficits on other tests of executive function and simple word retrieval (e.g naming, incomplete sentences) have been found.

Objectives: To investigate the contribution of language and executive dysfunction to the verbal fluency deficit.

Methods: Sixty-six ALS patients and 66 healthy controls in total (groups did not significantly differ in age, verbal IQ, education, anxiety and depression) underwent the Written Verbal Fluency Test (S words 5 mins, 4-letter C words 4 mins) modified for writing disability to produce the Written Verbal Fluency Index (Vfi), average time to 'think' of each word. Performance was subsequently analysed in terms of the production of clusters of semantically/phonetically related words, a process more reliant on language functions. In addition a detailed method for assessing the time taken to 'think' of a word within a cluster and to switch between clusters was developed, which was once again independent of writing speed. Whilst cluster formation reflects lexical associative networks (language functions), switching between clusters reflects generating a new strategy (executive functions). Hence it was predicted that if executive dysfunction underlies the verbal fluency deficit, ALS patients would show a disproportionate time to switch between clusters. Word thinking rates across the task were also calculated to assess initiation problems or fatigue.

Results: The ALS patients demonstrated normal cluster size indicating an intact associative language network, however both retrieval times (within a cluster and switch) were reduced. Patients also showed a reduced rate of word retrieval throughout the task with no initiation deficit or fatigue.

Conclusions: ALS patients have a normal pattern of letter fluency performance but have slowed 'thinking times' in all components of the test. The deficit does not appear to be due to executive dysfunction alone. The deficit may be caused by: A) combination of executive and language dysfunction, or B) generally slowed information processing. The implications in relation to subcortical and cortical dysfunction are discussed.

P221 WRITING ERROR IN JAPANESE ALS

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Keywords: writing error, frontal dysfunction, neuropsychological test

Background: Frontal lobe dysfunction is often seen in ALS patients. Clinically, some Japanese ALS patients are found to have difficulties in writing proper sentences when they came to need to write instead of speaking.

Objectives: Screening of frontal lobe function and writing ability was performed to clarify whether writing errors were a frequent symptom in non-demented ALS.

Methods: Eighteen ALS patients and 16 controls without a neurological disease were examined by Raven's progressive coloured matrices, trail making test, picture arrangements, some components of the Japanese version of Western Aphasia Battery (WAB IIC, III, IVB), reading, dictation and composition. To quantify and to analyze the writing errors statistically, we calculated "writing error rate (WER)" as a rate of sum of errors and total words they wrote. Patients with dementia, other neurological diseases and respiratory failure were excluded.

Results: Picture arrangements, auditory comprehension (WAB IIC), and kanji-dictation were significantly impaired in patients ($p = 0.0094$, 0.0157, 0.0219, respectively). In particular, WER on composition was much higher in the ALS group than in the controls (patients: $13.5 \pm 7.8\%$, control: $2.9 \pm 3.0\%$, $p = 0.0002$). Among various patterns of writing error, omission of kana-letters and loss of a subject were marked.

Discussion: Patterns of writing error like omission, substitution and displacement of kana-letter were often seen in Japanese patients having a lesion of the middle or inferior frontal gyrus. Writing error in ALS may represent frontal lobe dysfunction and was a specific symptom for these ALS patients even though they were not demented.

P222 FRONTAL ANOSOGNOSY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: anosognosy, frontal syndrome

Background: People affected by ALS present irremediable and progressive motor disorders. In addition cognitive impairment and also behavioral and psychological disturbances, which develop as a consequence of frontal lesions, are now recognised.

Objectives: To establish if these various cognitive, behavioral and psychological disorders of frontal origin can be related to a frontal anosognosy, known as supramodulaire.

Methods: Eighteen ALS patients, 10 men and 8 women (64.1 years $\pm$11.9), with a mean duration of 43 months ($\pm$7.5), and an average ALSFRS score of 38.9 and 18 control subjects, 10 men and 8 women (62.4 years $\pm$11.5) were evaluated with an anxiety scale (STAI), a depression scale (BDI-II), the neuropsychiatric inventory (NPI-R), a rating scale of the ignorance of the disorders (PCRS) and a battery of executive tests.

Results: The patients were less efficient than controls with the tests evaluating the working memory, mental flexibility and inhibition ($p = 0.0068$). The results were higher for the NPI-R in the ALS group ($p = 0.0002$). The latter were correlated with a lack of flexibility ($r = 0.666$; $p = 0.0018$). The BDI-II revealed weak scores for the two populations; however there was a significant difference ($p = 0.0445$). On the other hand, the PCRS did not reveal any sign of anosognosy ($p = 0.4765$).

Discussion and Conclusion: The main results show that the patients present cognitive disorders of frontal origin, as well as behavioral dysfunctions linked to a lack of mental flexibility. Depressive symptomatology is not sufficiently intense to speak about depression, but from a qualitative point of view, the patients appear distressed. The assumption that anosognosy can be related to the other functions is rejected.
Many disturbances of the psychological, behavioral and cognitive spheres exist, some of which are of frontal origin, but patients suffering from ALS are obviously not ignorant of the disorders.

P223 METHODOLOGICAL DIFFICULTIES WHEN ADHERING TO THE EMERGING CONSENSUS CRITERIA FOR ASSESSMENT OF COGNITIVE AND BEHAVIOURAL COMPONENTS OF AMYOTROPHIC LATERAL SCLEROSIS IN PRACTICE

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Keywords: psychometrics, consensus, critera

Background: We are currently conducting a longitudinal study in patients with ALS in order to characterise the cognitive and behavioural features of the disease in the Irish population. In doing so we are characterising the phenotypes of cognitive impairments in this patient group but have encountered significant methodological limitations of the current consensus criteria.

Objectives: To discuss the methodological difficulties encountered during the process of the study when attempting to apply the current ALS-FTD, ALSci and ALSbi consensus criteria in practice.

Methods: We will review the issues and difficulties under the following headings:
1) Psychometrics: A) Use of self-report measures: The use of self-report measures increases the risk of a number of errors which might result in an over- or under-estimate of cognitive-behavioural dysfunction. While such measures are helpful

they require careful use and might not be psychometrically sound in a research context. A further issue emerges when one uses the FrSBe patient-rated form. Such ratings might be unreliable due to the presence of cognitive deficit or reduced insight in the patient group. B) Statistical properties of measures: Detailed psychometric data are scarce for the FrSBe in ALS. Conclusions about behavioural change and cognitive deterioration are difficult and limited given the lack of extensive data on the form.
2) Criteria: A) Development of global index measures may not the most appropriate use of neuropsychological data. When trying to categorise patients by neuropsychological assessment, it is possible that we should be looking at deficits on individual neuropsychological test rather than trying to create global index measures. B) Behavioural descriptors: The FrSBe may be more sensitive to frontocortical areas and may miss behaviours associated with FTL degeneration like semantic dementia/compulsively stereotypic behaviours. C) Issues with solely using tests of executive functioning to categorise ALSci: Cognitive impairment criteria may be too narrow and theoretically invalid-the frontal-striatal system is broader than this.
3) Tests used: There are differences in ALS bulbar onset patients; alternate measures may be necessary. Development of computer assessments for such cases may be prudent.

Results and Discussion: We would like to generate an awareness of methodological issues with cognitive and behavioural assessment in ALS patients. Further discussion is necessary regarding the optimum methods for assessing ALS patients and gaining the most useful, reliable and objective information possible regarding behaviour, cognition and their relationship and overlap in ALS patients. There is a need for a more innovative approach to neuropsychological examination in ALS patients in light of emerging information regarding neurodegenerative processes in this unique population. Such endeavours will enable us to refine the information we obtain in order to achieve a more accurate understanding of this complex disease process.

P224 RELATIONSHIP BETWEEN BEHAVIORAL CHANGES AND COGNITIVE FUNCTION IN ALS

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Keywords: cognition, behavior, dementia

Background: Cognitive deficits in the context of ALS are widely recognized based on clinician judgment and neuropsychological tests, but less is known about the behavioral correlates of such deficits and their relationship to ALS severity and progression.

Objectives: To examine the association between behavioral changes and cognitive function and their relationship to indices of disease progression.

Methods: A neurologist completed the Clinical Dementia Rating Scale (CDR) and patients were administered a 1-hour battery of standard neuropsychological tests. Medical variables included the ALSFRS-R, time since symptom onset and site (bulbar or spinal) of onset. Caregivers rated patients on two behavioral measures: the Manchester Behavioral Questionnaire, which has 6 subscales and a total of 97 items, and the Frontal Behavioral Inventory (FBI) with 2 subscales and 24 items. The dependent variables that determined neurocognitive status were the CDR and a Deficit Score based on neuropsychological test performance. ANOVA tests analyzed the relationship between neurocognitive status and subscales as well as individual items from the behavioral tests. Multiple regression analyses assessed the relationships while controlling for medical variables.

Results: Forty patients with confirmed ALS participated. Mean age was 59 and the mean ALSFRS-R score was 25. Twenty percent of patients had bulbar-onset, and mean duration since symptom onset was 48 months. The subscale of Affective and Social Behavior from the Manchester Questionnaire was significantly associated with both measures of neurocognitive status, while the Repetitive Behaviors subscale was associated only with CDR scores. The total FBI score was associated with the CDR, but not neuropsychological test performance. The individual items of "loss of insight", "loss of interest", "loss of embarrassment" and "excessive worry" were all associated with both outcome measures. In multiple regression analyses, controlling for age, gender and clinical variables, only one of the 6 Manchester subscales (eating) and neither of the FBI subscales was associated with CDR stage, but the individual Manchester items of "loss of embarrassment," "loss of interest," and "high emotion" were all significant predictors (overall model ANOVA: $F = 9.78$, $p < 0.001$). Using the same covariates, the only significant predictor of Deficit Score was the Manchester single item, "loss of embarrassment" (Overall Model ANOVA: $F = 4.54$, $p = 0.004$).

Discussion and Conclusion: Several significant univariate correlations were observed between behavioral subscales and neurocognitive status. Multiple regression analyses, controlling for medical parameters, showed that individual items describing behavior changes were associated with neurocognitive changes, but that the subscale scores of the two behavioral measures were not. The overall pattern suggests that loss of interest and diminished social judgment are predominant behavioral characteristics of the cognitive deficit seen in ALS.

P225 RASCH VALIDATION OF THE HOSPITAL ANXIETY AND DEPRESSION SCALE IN MOTOR NEURONE DISEASE

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Keywords: HADS, Rasch, Item Response Theory

Objectives: To validate the scaling and psychometric properties of the HADS in MND using the Rasch measurement model.

Methods: 194 patients (average age 62.52 ± 11.11) from regional MND care centres in the UK completed the HADS either at home or during a routine clinic appointment. Two sets of Rasch analyses were performed on the HADS-D depression subscale and the HADS-A anxiety subscale.

Results: The HADS-A anxiety subscale displayed a good fit to the Rasch model (χ^2 (14) $= 17.22$ $p = 0.24$). Person separation

was shown to be sufficient to distinguish between individuals at a clinically significant level. One item "I feel restless as if I have to be on the move" displayed uniform response bias (DIF) between groups that filled out the questionnaire at home and in clinic. Category thresholds were collapsed for one item "I get a sort of frightened feeling like butterflies in my stomach" as response options "Occasionally" and "Quite Often" were indiscriminable. Independent T-test confirmed the unidimensionality of the HADS-A subscale.
The HADS-D subscale showed initial misfit to the Rasch model (χ^2 (14) = 158.72 $p < 0.05$). Fit to the model was improved following the removal of 3 poorly-fitting items "I still enjoy the things I used to enjoy", "I feel as if I am slowed down" and "I can still laugh and see the funny side of things". Disordered category thresholds were removed by collapsing indiscriminate response categories. The resultant 4-item scale displayed significant fit to the Rasch model (χ^2 (8) = 9.90 $p < 0.27$).

Discussion: The HADS-A proved a robust unidimensional measure of anxiety in this population. The HADS-D subscale did not fit the Rasch model due to extreme item misfit. The HADS-D requires further exploration to assess how patients interpret the items when responding to the questionnaire. It is recommended that when using the HADS to screen for depression in this population the modified version of the scale is used.

P226 ASSESSSING COPING OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS WITH THE FREIBURGER FRAGEBOGEN ZUR KRANKHEITSVERARBEITUNG

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Keywords: coping, depression, quality of life

Background: Amyotrophic lateral sclerosis (ALS) is still an incurable and lethal disease. However a lot of studies show that not all the patients with ALS get depressed, but report a satisfying quality of life. In trying to understand this phenomenon, we consider coping strategies as a possible explanation.

Objectives: To assess and evaluate coping strategies of patients with ALS.

Methods: Forty-seven patients with ALS completed the Freiburger Fragebogen zur Krankheitsverarbeitung in a short version (FKV-15) and answered questions regarding the feeling of being a burden, impairment and perceived control of ALS (visual analogue scale, range 1–10).

Results: The patients reached the highest values in the scales of active coping (M 3.63, SD 0.79) and compliance (M 3.97, SD 0.85), and the lowest values in the scales of depressive coping (M 2.63, SD 0.65) and minimising (M 2.47, SD 0.89). Significant correlations were found between depressive coping and compliance ($r = 0.31^{*}$) as well as between depressive coping and the perceived control of ALS ($r = 0.36^{*}$); furthermore between compliance and active coping ($r = 0.44^{**}$). The feeling of being a burden for the family correlates positively with the impairment through ALS ($r = 0.29^{*}$) and negatively with compliance ($r = -0.40^{**}$).

Discussion and Conclusion: Active coping, meaning focussing on problems and looking for information is one of the most adopted strategies of our patients. The correlation between this strategy and compliance reveals an influence of the factors on each other. It is possible that persons focussing on problems and being interested in getting information are more compliant – or vice versa. Moreover a negative correlation was found between compliance and the feeling of being a burden. This suggests that we should strengthen strategies of patients that increase compliance because it may thereby be possible to reduce the very stressful feeling of being a burden. However all of these first results cannot explain why most of the patients with ALS (in spite of the restrictions accompanying ALS) seem to be satisfied with their life. Possible explanations may be gained considering the "well-being paradox" (1, 2) or the "response shift" (3). In the future we would like to assess whether one of these concepts qualifies for patients with ALS and helps to explain to a greater extent the actual results in this field.

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P227 NEUROPSYCHOLOGICAL DEFICITS IN AMYOTROPHIC LATERAL SCLEROSIS-A SOUTH INDIA EXPERIENCE

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Keywords: cognitive deficits, executive function

Background: Amyotrophic lateral sclerosis (ALS) is a terminal neurological disorder characterized by progressive degeneration of nerve cells in the spinal cord and brain. Cognitive impairment was not identified in the ALS population until fairly recently. Studies suggest that approximately 35% to 52% of ALS patients experience cognitive deficits which may be identified early in the course of the disease. Cognitive deficits as an integral part of the disease have not been studied in the Indian setting. This is one of the first studies assessing the pattern of cognitive impairment in ALS in the Indian condition.

Objective: To determine the profile of cognitive impairment in patients diagnosed with ALS.

Methods: Cognitive function was studied in 20 patients (mean age 45.85 ± 13.9 years (22–65)) with confirmed ALS. A neuropsychological test battery comprising tests for attention and executive functions as well as verbal and visual learning memory was administered. In all 21 tests were administered individually in 4–5 sessions which lasted for 7–8 hours.

Results: The majority of patients were from lower/middle socio-economic backgrounds. All patients were right handed. Scores were compared with gender, age and education specific norms, wherein scores falling below 15th percentile of the normative data were treated as deficits. ALS-associated

cognitive impairments include deficiencies in visual attention, working memory, cognitive flexibility, response inhibition, planning, problem solving, visual-perceptual skills and intrinsic response generation, i.e. verbal fluency independent of dysarthria. Fluency deficits are not limited to verbal abilities but also non-verbal fluency (designs), supporting the notion of an underlying deficit of response generation. These impairments indicate executive dysfunction.

Conclusion: ALS is a disease that affects higher cognitive frontal functions, especially the executive functions.

P228 PRAGMATIC COMMUNICATION SKILLS IN ALS

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Keywords: communication, cognition

Background: Pragmatic communication refers to the social aspects of language, including topic selection and maintenance, turn-taking, paralinguistic abilities and also non-verbal skills. Competence in such areas enables effective social interaction and impairments can cause misinterpretation and break down of understanding. The decline in physical functioning in ALS can lead to impairments in specific aspects of pragmatic communication (prosodics, use of gesture, facial expression and control of physical proximity). In addition, executive dysfunction, present in approximately one third of patients with ALS, may also impact upon pragmatic communication as is the case in other neurological disorders such as frontotemporal dementia and Parkinson's disease.

Objectives: To investigate whether ALS patients show deficits in pragmatic communication and explore the relationship between impairments in this domain and executive dysfunction.

Methods: Thirty-two patients with ALS and 20 healthy controls completed tests of pragmatic communication skills and executive functioning. The study used the Prutting and Kirchner (1983) pragmatic protocol to produce a Pragmatic Communications Questionnaire (PCQ). Twenty-four statements concerning pragmatic communication abilities were generated to cover seven key areas of pragmatic communication. Each statement was read to the participant who was asked to rate their level of agreement with the statement (strongly agree (0), moderately agree (1), moderately disagree (2) and strongly disagree (3)). High scores indicated inappropriate pragmatic skills. Spouses, or carers who knew the patient well, were also asked to rate the patients' abilities using an adapted version of the PCQ. The ratings were obtained on two separate occasions and a mean rating score was generated. Executive functioning was assessed using Thurstone's Written Verbal Fluency with adjustment for motor impairment. Participants were requested to generate words beginning with S in 5 minutes and four letter words beginning with C in four minutes and Verbal Fluency Index (VFI) scores were generated for each letter. Physical function was assessed using the Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised (ALSFRS-R).

Results: Analysis revealed that ALS patients have significant impairments in self-rated pragmatic communication skills compared to controls, with a significantly higher mean score on the PCQ. This impairment was related to degree of executive function as measured by verbal fluency. After controlling for levels of physical disability (ALSFRS-R scores), there was a significant partial correlation ($p < 0.05$) between VFI scores and self-rated PCQ scores, with higher (i.e. more impaired) VFI scores being associated with poorer pragmatic communication. There were significant partial correlations ($p < 0.05$) between the patients' VFI scores and the PCQ ratings obtained from their spouse/carer.

Conclusion: This study demonstrated evidence of impairments in pragmatic communication in ALS and suggests that, as in other neurodegenerative disorders, this impairment may be related to executive dysfunction.

P229 OCCURRENCE OF THE "APPLAUSE SIGN" IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: applause sign, frontotemporal dementia, EXIT-25

Background: The three clap test ("applause sign") has been described as a quick bedside test to discriminate progressive supranuclear palsy (PSP) (positive applause sign) from Parkinson's disease and frontotemporal dementia (FTD) (negative applause sign). The anatomical and pathophysiological basis of the applause sign is enigmatic. However, it has been hypothesized that a combined dysfunction of the frontal lobe and the basal ganglia may underlie this phenomenon.

Objectives: To address the question of whether a positive applause sign may also occur in ALS patients. Furthermore, to perform neuropsychological testing to correlate the occurrence of a positive applause sign with specific neuropsychological symptoms, especially frontal lobe dysfunction.

Methods: Twenty-two patients with ALS were matched with 22 healthy controls. Patients and controls were tested for the occurrence of the applause sign. To asses the "three clap test", the patient is asked to clap three times as quickly as possible, but only three times, after demonstration by the examiner. The performance of the subject is normal when he or she claps only three times or abnormal if he or she claps more than three times (positive applause sign). Neuropsychological testing included the Mini Mental State Exam (MMSE) to evaluate general cognitive functions in patients and controls and the EXIT-25-test to detect specific frontal dysexecutive symptoms.

Results: Five ALS patients (22.7%) and none of the controls displayed a positive applause sign. Two of the patients with positive applause sign had pathologic scores in the EXIT-25 and one was demented according to MMSE scores. Thus, the occurrence of the applause sign was not correlated to EXIT-25 or MMSE scores. Furthermore, the applause sign was not correlated to the severity of the disease as assessed by the ALS- functional rating scale or bulbar involvement, nor was it related to the duration of the disease.

Discussion and Conclusions: The results demonstrate that the applause sign occurs in a subset of ALS patients and is not specific for PSP. It does not correlate with the occurrence of dysexecutive symptoms as assessed by the EXIT-25 test. Therefore, pure frontal dysfunction may not be sufficient to evoke a positive applause sign. This underlines the original hypothesis that frontal deficits combined with subcortical dysfunction may be necessary for a pathologic test. This may fit well into the ALS pathology, since impairment of frontal functions or overt FTD is observed in up to 35–50% of patients and basal ganglia involvement is evidenced by various radiological and histopathological studies.

P230 PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS SHOW CHARACTERISTIC PERSONALITY TRAITS

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Keywords: personality, proneness to distress, NEO-Five Factor Inventory

Background: Amyotrophic Lateral Sclerosis (ALS) is a relentlessly progressive disease characterized by the death of upper and lower motoneurons. The pathogenic basis of this deeply disabling disease is still unclear. For several decades clinicians have investigated whether a distinctive premorbid personality is a feature in neurodegenerative disorders, such as Parkinson's disease. Recently, it was demonstrated that a predisposition to experiencing negative emotion or distress can be associated with an increased risk of the incidence of Alzheimer's Disease and mild cognitive impairment. A recent report suggested that patients with ALS may show specific premorbid personality characteristics, such as exerting control over their emotions, preferring familiarity and routine and being conventional in behaviour and conservative in outlook. Furthermore, the complex relationship between premorbid personality and ALS is fuelled by anecdotal reports by patients and caregivers suggesting that stressful events often preceded disease onset.

Objectives: To analyse the personality traits of ALS patients.

Methods: We present here preliminary results concerning the first 33 ALS patients (6 with bulbar onset, 27 with spinal onset) enrolled at our tertiary referral centre. The mean age was 58.12 years, with 10.73 years of education. Clinical characteristics of the patients were recorded. The control group of healthy subjects was strictly paired for demographic characteristics. All the participants in the study underwent a standardized measure of five major domains of personality: N, neuroticism; E, extraversion; O, openness; A, agreeableness; C, consciousness, evaluated through the NEO-Five-Factor Inventory (NEO-FFI). The raw scores of the NEO-FFI are summarized in terms of five levels: very low, low, average, high and very high, on the basis of the equivalent score (T score) obtained. Means and standard deviations of T scores for patients and controls were calculated and analysis of variance (ANOVA) was performed.

Results: The personality traits assessed by NEO-FFI showed significantly lower openness scores for the ALS patients (ALS, 47.9 ± 11.4) vs controls (54.4 ± 9.9, $p < 0.02$). With regard to the other traits, no significant differences were found between patients and controls. However, ALS patients scored higher at the N domain, suggesting that these patients might actually be prone to distress.

Discussion and Conclusion: We show here that ALS patients may bear a specific personality profile, with low openness, a propensity for keeping their mind on the tasks at hands and exerting control over their emotions. The high score in N domain suggests that ALS patients tend to experience negative emotions and are prone to distress. We are currently studying the possible impact of this personality type on management and end-of-life issues.

P231 RECOGNITION OF FACIAL BASIC AND SOCIAL EMOTIONS IN ALS WITH AND WITHOUT FRONTOTEMPORAL DEMENTIA

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Keywords: facial expressions, frontotemporal dementia, bulbar onset

Background: Previous studies have indicated that ALS patients have disturbances in emotional perception and memory. Disturbances in emotional recognition may reduce patients' involvement in diverse relationships and activities.

Aims: To assess the recognition of facial expression of primary and social emotions and to correlate it with frontotemporal impairment in a series of ALS patients.

Methods: A total of 43 patients with definite or probable ALS and 43 healthy age- and gender-matched controls were tested. Patients and controls underwent the Ekman 60 Faces test in the Facial Expression of Emotion-Stimuli and Tests (FEEST), that measures recognition of facial expression using 6 different basic emotions (fear, anger, surprise, disgust, sadness, happiness, 10 of each). They were also assessed with the social emotion section of FEEST using 2 different social emotions and neutral expressions (arrogance, flirtatiousness, neutral, 10 of each). Patients and controls were also administered the Hospital Anxiety and Depression Scale (HADS). In order to evaluate frontotemporal impairment, patients underwent the Frontal Systems Behaviour Scale (FrSBe) and the Category and Letter Fluency Tasks.

Results: The patients correctly identified significantly less basic emotions than healthy controls (45.8 vs. 51.5; $p = 0.007$). Significant differences were found for sadness, surprise, disgust and happiness. Twelve patients (27.9%) had performances under the cut-off score of the Ekman 60 faces test. Conversely, the recognition of social emotions was similar in patients and controls. The correct identification of facial emotions was not related to patients' or controls' anxiety or depression. The presence of bulbar signs at the time of the evaluation did not influence the recognition of basic and social face expression. Twenty patients (46.5%) had a mild behavioural and/or cognitive frontotemporal dementia (FTLD).

The presence of FTLD was not related to the identification of either basic or social emotions, although patients with frontotemporal impairment had worse performances in all tests.

Conclusions: Emotional recognition deficits occur in bulbar ALS, particularly with emotional facial expressions, and can arise independently from depressive and frontotemporal symptoms. These findings indicate that the recognition of emotional expressions is impaired in ALS indicating that ALS is a multisystem disorder involving cognitive as well as motor deficits.

P232 MOTIVATION DISORDERS IN PROGRESSIVE NEUROLOGICAL DISEASES: APATHY IN AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSON'S DISEASE

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Keywords: apathy, motivation, cognition

Background: Apathy is a motivation disorder that has an important influence in the emotional domain. Studies published concerning the relationship between apathy, cognitive and emotional disorders are not exhaustive. No comparisons have been established according to the different kinds of neurological diseases.

Objectives: To demonstrate the heterogeneity of the apathetic syndrome and its mechanisms in different progressive neurological diseases (Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS)) and to analyze the relationship between apathy, cognitive and emotional disturbances. Motivation disturbances were also studied using a new scale developed by Derouesné *et al* (2004) that allowed a qualitative analysis of motivation.

Method: Twenty-one ALS patients, 20 Parkinsonian patients and 36 control subjects were assessed using neuropsychological and psychoaffective tests.

Results: The results agree with previous studies and showed the heterogeneity of apathy in the two studied diseases. This heterogeneity is explained by the relationship of the apathy with the emotional data but not by the cognitive data. No differences were found in cognitive performances between the two groups of patients. ALS patients showed higher scores for irritability and hypoexpressivity. Concerning apathy and motivation, ALS patients showed higher scores for the Apathy Evaluation Scale than Parkinsonian patients but in the new qualitative motivation scale, ALS patients attributed the reduction of activities to the motor handicap induced by the disease whereas Parkinsonian patients attributed the reduction of activities to a real lack of motivation.

Conclusions: The qualitative analysis of motivation allowed a better understanding of the apathy mechanisms and showed that patients with Parkinson's disease and ALS have different profiles of apathy and they attribute it to different causes. Apathy needs to be assessed with a descriptive and qualitative scale that considers patients with motor impairment.

P233 EXPLAINING APATHY IN ALS

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Keywords: apathy, depression, neurovegetative

Background: Apathy is one of the more common behavioral alterations associated with ALS. Apathy is a multidimensional construct and it is unclear if its relatively high frequency reflects frontal lobe pathology, depression, fatigue, physical weakness, or a coping mechanism for dealing with terminal disease.

Objectives: To examine the association between ratings of apathy and various factors that could lead to apathy in a non-demented ALS cohort.

Methods: Twenty-six patients with ALS underwent testing. Apathy was determined using the Self and Caregiver Rating Forms from the Frontal Systems Behavioral Scale (FrSBe). Functional disability was measured using the ALSFRS-R. Neurovegetative symptoms of fatigue and weakness were assessed with the Chicago-Multiscale Depression Inventory (CMDI), a novel measure developed specifically for use with medical patients that provides standardized scores for three domains of depression: mood symptoms (i.e. sadness, hopelessness), evaluative symptoms (i.e. self-criticism, guilt or shame) and vegetative symptoms (i.e. fatigue, weakness, sleep disturbance). Subjects also completed the Beck Depression Inventory (2^{nd} ed.) (BDI-II). Correlations were completed using Pearson's r correlation analyses.

Results: Our patients with ALS reported significant levels of current apathy (93^{rd} percentile compared to a normal population). Family members rated the patients as elevated in terms of apathy, but to a lesser degree (84^{th} percentile). Six of the twenty-six patients endorsed enough symptoms of depression to raise clinical suspicion of at least a moderate level of depression. Self-ratings of apathy showed a mild correlation with vegetative symptoms ($r = 0.30$), while family ratings of patient apathy were not correlated with vegetative symptoms ($r = 0.07$). Stronger correlations were seen between apathy and mood symptoms ($r = 0.58$) and total BDI-II scores ($r = 0.613$). We found no correlation between apathy and speech rate ($r = -0.04$), duration of symptoms ($r = -0.06$), or FVC ($r = -0.06$). There was a weak correlation between self-reported apathy and ALSFRS-R scores ($r = -0.26$).

Discussion and Conclusions: Symptoms of apathy in ALS do not appear to be strongly associated with fatigue, respiratory insufficiency, or neurovegetative symptoms. In contrast, apathy has its strongest correlation with alterations in mood, yet apathy often occurs in a setting where clinically significant depression is not diagnosed. In this construct, patients with apathy may deny overt feelings of depression or hopelessness, but are still likely to manifest symptoms of an underlying disorder that might be amenable to therapies directed toward coping mechanisms and mood.

P234 FATIGUE IN MOTOR NEURONE DISEASE: ITS RELATIONSHIP WITH DEPRESSION AND ANXIETY

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Keywords: HADS, depression, anxiety

Background: Fatigue is frequently reported in people with MND but it is unclear whether this relates to demographic variables such as age and gender, or whether such symptoms are related to other more specific mood states such as depression.

Objectives: To evaluate the relationship between fatigue and other variables in MND such as depression, anxiety, age and sex.

Methods: 154 patients (average age 61.9 ± 10.62) from five regional MND care centres in the U.K. completed questionnaires either at home or during a routine clinic appointment. Fatigue scores were taken from a recently developed MND fatigue scale and a single-item visual analogue scale (VAS) of fatigue. Depression and anxiety were measured using the Hospital Anxiety and Depression Scale (HADS).

Results: Pearson's correlation analysis revealed that the scale scores for fatigue had no direct relationship with depression ($r = -0.123$ $p = 0.15$) or anxiety ($r = 0.096$ $p = 0.27$) and a weak negative relationship with age ($r = -0.194$ $p = 0.02$). The fatigue scale scores were positively related to a fatigue assessed with the simple VAS ($r = 0.173$ $p = 0.04$). T-tests revealed no gender differences in fatigue ($t = -0.22$, $df = 140$ $p = 0.83$) or depression ($t = -0.34$, $df = 140$, $p = 0.73$) but higher anxiety scores were recorded in women ($t = -2.225$, $df = 140$, $p = 0.03$). 42.3% of participants scored 11+ on the HADS depression sub-scale, suggesting case-level depression. Median scores of fatigue were no higher in the case-level subgroup than those that scored below 11 on the HADS-D subscale.

Discussion: The results are congruent with other recent studies that indicate fatigue in MND is unrelated to depression or anxiety and may be more prevalent in younger patients. The high prevalence of case-level depression revealed by the HADS scores may be accounted for by the scale's high sensitivity and potential confounding between physical symptoms in MND and scale items such as "I feel slowed down" and "I still enjoy the things I used to enjoy".

P235 ALS PATIENTS: MAYBE DEPRESSED, SURELY NOT ANXIOUS

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Keywords: depression, anxiety, caregivers

Background: A lot of studies have investigated psychological aspects in people with Amyotrophic Lateral Sclerosis (ALS) but there is still uncertainty about the real morbidity, in particular for anxiety.

Objectives: To evaluate anxiety and depression levels in ALS patients and their caregivers, in comparison with healthy controls.

Methods: Thirty ALS patients, their caregivers and 30 healthy adult subjects completed Beck Depression Inventory II (BDI-II) and State-Trait Anxiety Inventory-Y2 scale (STAI). We compared overall and single item scores, in order to find differences between the three groups.

Results: BDI-II scores were significantly different between groups. Depression scores were higher in patients than in healthy controls, for both somatic and psychological subscales. Caregivers presented higher levels of psychological depression in comparison with healthy controls and lower scores of somatic depression in comparison with patients. No difference was found on anxiety levels comparing the three groups.

Discussion and Conclusion: ALS patients and their caregivers developed more depression-related symptomatology than the non-clinical sample. However, anxiety levels of patients and caregivers seem to be normal.

P236 NEUROBEHAVIORAL SYMPTOMS IN ALS: DIFFERENCES BETWEEN PATIENT AND CAREGIVER PERCEPTION

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Keywords: FTLD, neurobehavioral symptoms, FrSBe

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive weakness involving upper and lower limbs, bulbar and respiratory functions. Commonly, cognition in ALS is considered as spared. There are now indications that 10 to 50% of ALS patients present a cognitive decline from subtle impairment to an overt frontotemporal lobar dementia (FTLD) characterized by personality changes, irritability, poor insight and deficit in frontal executive tests.

Objectives: To evaluate neurobehavioral symptoms in a series of ALS patients and compare their perceptions with those of caregivers; to correlate neurobehavioral symptoms to functional status.

Methods: A total of 68 consecutive ALS patients (mean age 64.07; 43 males) and their primary caregivers were enrolled in 2 ALS centres (Turin University, Maugeri Foundation Milan). Patients were administered: 1) Frontal Systems Behaviour scale (FrSBe), a self-administered scale with a total score and 3 sub-scores for each domain: apathy, disinhibition and executive dysfunction, reporting patient perception before and after ALS onset; 2) category and letter fluency tasks; 3) Mini Mental State Examination (MMSE) and 4) Cambridge Behavioural Inventory (CBI). Functional status was assessed with ALS Functional Rating Scale (ALS-FRS). Caregivers were administered FrSBe and CBI. Correlations between variables were evaluated with Pearson's correlation coefficient; comparison between means was evaluated with Student T-Test (SPSS 12.0, $p < 0.05$; two-sided).

Results: No patient was normal on all tests. Four patients had a score under the cut-off (<23.4) for cognitive impairment and 16 patients had mild cognitive impairment (score between 23.4 and 26.5). Ten patients had pathological sub-score for letter fluency tasks and 7 patients for category fluency tasks. Four patients were diagnosed as definite FTLD. In patients mean FrSBe total score increased from 47.5 to 58.1 before and after ALS onset (27 pathological). According to caregivers mean FrSBe scores were respectively 53.6 and 63.6 (34 pathological). The differences between patients and caregivers were not significant. Thirty-eight patients had pathological sub-score for apathy, 16 for disinhibition and 24 for executive dysfunction. The apathy scale was the most commonly impaired (from 7.4% to 42.6% according to patients; from 16.2% to 45.6% according to caregivers). The presence of bulbar symptoms was significantly correlated to FrSBe and CBI total scores ($p < 0.01$).

Conclusions: According to caregivers, about half of the patients showed neurobehavioral symptoms after ALS onset; a lower number of patients were aware of the presence of neurobehavioral disturbances. The most frequent behavioural symptom was apathy.

P237 CAREGIVER BURDEN IN MND: THE ROLE OF COGNITIVE AND BEHAVIOURAL IMPAIRMENT

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Keywords: caregiver, subjective, stressors

Objectives: Executive dysfunction and behaviour impairments have been reported in a proportion of people with Motor Neuron Disease (MND). This study investigated the impact of patients' behaviour and executive dysfunction on caregivers' reported psychological distress. Objective and carer-rated measures of executive function were investigated. The relationship between objective and subjective stressors and their respective impact on caregiver distress was examined.

Methods: Thirty-two caregivers completed self-report measures of mood, quality of life and caregiver burden. Caregivers and patients completed a measure of patients' apathy, disinhibition and executive function (FrSBe). Objective measures of executive function (Verbal fluency, the Stroop Test and Brixton Spatial Anticipation Test) were completed with the patient as part of a population based study of cognitive decline in MND.

Results: Scores on objective executive dysfunction measures did not significantly predict caregiver burden or mood. However, caregivers' subjective ratings of patients' behaviour change significantly predicted caregiver burden, anxiety and depression.

Discussion and Conclusions: This study suggests that the burden, anxiety and depression experienced by caregivers are influenced by their perception of changes in behaviour of the patient. These findings suggest that behavioural and cognitive impairment in ALS would constitute an important facet of a psycho-educational intervention for caregivers.

P238 PHENOMENON OF "SELF-RECLAMATION" FOUND IN LONG-TERM ALS FAMILY CAREGIVERS

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Keywords: caregiver, narrative

Background: It has become possible to prolong the life of ALS patients by applying ventilation, and at the same time, this has led to prolonged care. Even so, there have been few research projects clarifying the actual conditions of ALS care especially for caregivers with more than ten years experience. We have reported on a phenomenon of "synchronized identification" between ALS patients and their family caregivers and that after the death of the patients, the phenomenon of "fragmented memories" occurred in family caregivers. In this research, it was clarified that in order for the ALS caregivers to continue caring, it is necessary to undergo a process of "self-reclamation".

Objectives: To clarify how family caregivers have positioned the ALS care in their lives and contrived the methods of caring.

Methods: This study used a qualitative descriptive method involving caregivers' narratives to investigate how family caregivers have been caring for ALS patients for long periods.

Results: There were 12 spouses and their experience of caregiving ranged from 2–16 years. The mean patients' ALS history was 9.8 years. There were nine who used a ventilator. As a result of the analysis, it was revealed that along with the progression of symptoms, the relationship between ALS patients and their caregivers intensified. In the case of the patients using ventilators, their caregivers said that they remained awake naturally even after midnight when the patients had sputum sticking in their throats and said, "It is very strange because I feel as if there is something connecting us." Therefore, for the caregivers to continue caregiving effectively, they had to get rid of this intensified relationship. This is the operation of "self-reclamation". The most important concern for caregivers was to find a meaningful life for their patients in addition to acquiring appropriate caregiving techniques. In order to accomplish this, it is important for the caregivers to have some additional breathing room in their lives. "Self-reclamation" was performed in the form of role-play within the family at first. The result was that by setting up time to be alone, the caregivers made efforts, for example, to take a walk, garden or do something else to leave the bedside even if for just 30 minutes. Furthermore, the caregivers made efforts to rediscover their identity in society by participating in a patients' association and communicating with others.

Conclusions: ALS caregivers tend to prioritize their patients over themselves and not realize their chronic stress. From now on, it is expected that they will have opportunities to exchange information among family caregivers, learn effective care methods from a professional standpoint and develop support programs including consultation services over the internet.

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THEME 10 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P239 EFFECT OF INSULIN SUPPLEMENTATION ON BODY COMPOSITION AND HAND GRIP MUSCLE FORCE IN ALS PATIENTS

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Keywords: body composition, fatigue index, muscle force

Background: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurological disease caused by the degeneration of motor neurons. The appearance of so-called "bulbar" weakness can occur at any time during the clinical course of ALS and carries an adverse prognostic implication for patients' survival. Surprisingly, the nutritional consequences of ALS have received poor attention. Some authors found that 70% of ambulatory ALS patients consumed less energy than the recommended dietary allowance based on 24h dietary recall (1). The reduction in energy intake correlated with increased weight loss, the degree of dysphagia reported by the patient, and a reduction in muscle wasting and triceps-skinfold thickness. Most authors recommend nutritional supplementation only after dysphagia and weight loss become prominent (2).

Objectives: We evaluated the effects of insulin supplementation in ALS patients on body composition (BIA method), muscle force fatigue index (20 sec^{-1} fatigue hand grip force/time curve), arm muscle areas (AMA) and nitrogen balance.

Results: Twelve non-obese women with ALS were recruited for this study. Patients were staged clinically according to the Norris Scale. During the week immediately preceding the study, each patient recorded food intake with the assistance of a digital photography system. Seven patients received 0.3 $U.I.Kg^{-1}$ crystalline zinc insulin s.c. infusion three times a week (insulin group, IG), five patients no additional insulin (control group, CG). BIA analysis system (RLJ-system inc. Detroit), nitrogen balance, muscle hand grip force/time, AMA (3) were assessed at the 60^{th}, 120^{th} and 180^{th} day. Data were analysed at the baseline and at the 180^{th} day. BIA and triceps-skinfold thickness by Desport's equations in ALS patients (4) were used for FFM_{50Hz}: there was a decrement in all groups, but less in the IG (4.8%: 44.0 ± 9.1 vs 42.1 ± 7.8 Kg) than in the CG (10.6%: 44.6 ± 9.8 vs 39.7 ± 7.5). Changes in the fatigue index were significantly ($p = 0.05$) higher in the CG than in the IG (5.1 vs 3.7 with polynomial 2^{nd} degree index). AMA showed no significant decrements in IG (24.2 ± 5.6 vs 23.9 ± 4.7 cm^2), but in the CG muscle loss was 25 ± 3.7 vs 21.2 ± 2.2 cm^2 (at the end of the protocol). No significant changes in 24-h nitrogen balance were found in both group (CG $<8\%$ and IG $<10\%$).

Conclusion: Our data showed that insulin supplementation is somehow able to counteract muscle mass and functional deterioration over time in ALS patients.

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P240 LEPTIN AND GHRELIN SERUM CONCENTRATIONS IN PATIENTS WITH ALS

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Keywords: leptin, ghrelin, T lymphocytes

Background: Leptin, an adipocyte-derived hormone of the cytokine family and ghrelin, a gastric-derived hormone, represent a link between body energy metabolism, nutritional status and immune and inflammatory response. Leptin promotes CD4+ T cell proliferation, whilst it can act as a negative signal for the expansion of human naturally occurring CD4+CD25+ T regulatory (Treg) cells. Ghrelin inhibits release of pro-inflammatory cytokines from LPS-stimulated macrophages. ALS patients are known to develop changes in their nutritional status and alterations in their metabolism; moreover, important changes occur in their innate or adaptive immune responses.

Objectives: In this study we examined the serum levels of leptin and ghrelin in a cohort of ALS patients with reference to their nutritional status and disease severity. In addition, we analysed a possible correlation between the percentages of CD4+ T lymphocytes, Treg cells and leptin serum levels.

Methods: Serum leptin and ghrelin levels were measured by immuno-ELISA assay in 41 ALS patients and 40 healthy donors. We also collected data on nutritional status using bioelectrical impedance analysis. Nutritional evaluation included: body weight, body mass index, fat mass, fat free mass.

Results: Serum leptin is proportional to the body fat mass in both genders of ALS patients and controls. However, although both males and females with ALS showed higher body fat mass compared to the healthy controls, only females with ALS exhibited remarkably and significantly higher serum leptin concentrations than respective controls. Serum ghrelin, which as expected is higher in females than in males, did not differ between ALS patients and healthy controls or correlate with body fat mass in ALS patients and controls of both genders. No significant correlations were found between serum leptin or ghrelin concentrations and disease duration and progression rate, or with the ALSFRS-R score in all patients.

Discussion and Conclusions: Body fat mass increases in ALS patients as consequence of muscle mass reduction. Although the serum leptin levels are proportional to the fat mass in both ALS patients and controls, the ALS females exhibit much higher circulating leptin than the other groups.

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Whether such effect has possible implications for the immune-inflammatory response in ALS females remains to be elucidated.

P241 NUTRITIONAL MANAGEMENT AND SURVIVAL IN ALS PATIENTS

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Keywords: PEG, bulbar onset, nutritional problems

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by a selective loss of motor neurons leading to progressive muscle weakness and muscle atrophy. ALS patients develop dysphagia and have nutritional problems. Recently, it has been reported that early percutaneous endoscopic gastrostomy (PEG) placement is beneficial for ALS patients with bulbar palsy.

Objectives: ALS patients with nutritional problems were provided with information about how early PEG placement might be beneficial. Some patients still hesitated to go ahead with the PEG, and sometimes the opportunity passed because of the progress of respiratory failure. In this study, the correlation of survival with PEG placement was analysed.

Methods: We evaluated 33 consecutive ALS patients with nutritional problems (22 men and 11 women; mean age $\pm$SD: 68.0 $\pm$ 13.1 years (yrs), 21 spinal onset and 12 bulbar onset) from April 2006 to April 2008. We followed their survival for one year after the day when patients, together with their practitioners, made the decision on how to manage nutritional problems.

Results: Seventeen patients (7 spinal onset and 10 bulbar onset) chose PEG placement, and the others (14 spinal onset and 2 bulbar onset) chose to receive nutrition via nasal tube feeding or intravenous drip (non-PEG). The average age of those who chose the PEG was 65.6 yrs, while the average age of non-PEG was 70.6 yrs. One year later, 14 of the 17 PEG patients were living without ventilator support, while 10 of the 16 non-PEG patients had died or needed ventilator support.

Discussion and Conclusion: Our study supports the benefit of PEG replacement for ALS patients with bulbar palsy. The difference in age and onset type of the patients must be considered.

P242 GUIDELINE FOR NUTRITIONAL CARE IN ALS

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Keywords: guideline, nutritional care, dieticians

Background: Most Dutch multidisciplinary ALS treatment teams have implemented the protocol for rehabilitative management in ALS (1). Although the ability to eat and drink are two of the most important functions in life and the progressive loss of these abilities has an immense impact on the patients and their families, the dietician was not always an integral member of the treatment team (2). It is known that optimal nutritional management by a dietician can positively influence survival and quality of life, but detailed evidence-based information for dieticians is lacking.

Objectives: To obtain a nutritional guideline in order to equip dieticians in helping to provide optimal nutritional care for ALS patients.

Methods: A guideline has been developed by reviewing the literature, in combination with practice-based experience from ALS dieticians. The guideline (3) has been written by Dieticians for Neuromuscular Diseases, an officially registered group of the Dutch Association of Dieticians.

Results: The guideline was published in the autumn of 2007. It is the initial step toward developing a national nutritional policy, for which the implementation continues.

Discussion and Conclusions: The guideline is a an instrument to initiate national and international discussion considering the statements of preventing weight gain, energy requirements influenced by progression and weight evolution, protein requirements, use of fibres during the course of the disease and defining palliative nutrition. Presentation of the statements will lead to discussion and establishing consensus on the nutritional requirements of ALS patients.

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P243 NUTRITIONAL PROGNOSTIC FACTORS IN AMYOTROPHIC LATERAL SCLEROSIS

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Rationale: It is well-known that hypermetabolism, weight loss, and cachexia are common phenomena in Amyotrophic Lateral Sclerosis (ALS) which contribute to prognosis. In this study we investigated the influence of Body Mass Index (BMI), serum glucose, serum cholesterol (LDL, HDL and LDL/HDL-ratio) and triglyceride levels on survival.

Methods: Over a time period of 10 years we prospectively and retrospectively investigated the course of disease of 419 patients (age = 58.4 $\pm$ 12.1 years). Among others we collected survival data, clinical scores and laboratory values, including nutritional factors, were collected. Cholesterol and triglyceride parameters were collected at time of diagnosis.

Results: We found that high serum levels of both cholesterol and triglycerides had a positive effect on survival ($p < 0.05$). We found a mean prolonged life expectancy of 21.6 months for patients with a serum triglyceride level $>$177 mg/dl. There was a highly significant correlation between triglyceride level and BMI ($p < 0.001$). We found that 11.8% of the subjects had a hyperglycemia, and 9.7% had a manifest diabetes mellitus which is above-average.

Conclusions: The results suggest that high triglyceride and cholesterol levels might be beneficial for ALS patients. Therefore, a diet rich in calories and lipids can possibly contribute to a favourable course of disease. The prescription of statins should be approached cautiously although further studies are necessary in this regard.

P244 ALS-CACHEXIA IS AN EARLY SIGN OF MOTOR NEURON DISEASE WITH TRUNCAL ONSET – RESULTS FROM A RETROSPECTIVE CLINICAL STUDY

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Keywords: ALS cachexia, truncal onset, ventilatory insufficiency

Background: Significant weight loss is common in amyotrophic lateral sclerosis with bulbar onset and in late stages of motor neuron disease. In patients without bulbar onset, it rarely occurs in the initial stages of the disease. F. Norris used the term ALS cachexia for severe early weight loss in patients with nonbulbar disease and considered it a poor prognostic sign.

Objectives: The purpose of this study was to identify clinical characteristics of patients with early severe weight loss in nonbulbar motor neuron disease and compare them to patients with bulbar and other nonbulbar disease.

Methods: In a retrospective clinical study we screened all ALS patients presenting to our clinic during a 2 year period with a disease duration of less than 26 months. Seventy-three patients were included, for whom weight and spirometry data were available.

Results: The study population comprised 45 patients with limb onset and 21 patients with bulbar onset. Seven patients presented with truncal onset, both with weakness of the paravertebral muscles and with early weakness of the respiratory musculature. In patients with truncal onset, vital capacity was reduced to a mean of 50.3% (minimun 33%, maximum 72%). Capillary blood gas analysis showed carbon dioxide retention (mean 55.7%).
All patients with truncal onset suffered from significant weight loss amounting to a mean of $19.9 \pm 7.1\%$ of the body mass index (BMI). Marked weight loss resulted in an extended tumor search in two of the seven patients prior to the diagnosis of motor neuron disease. None of the seven patients had significant bulbar involvement during their initial evaluation in our institution. Patients frequently complained of an early satiety feeling which limited their caloric intake. In some patients, the early weight loss was partially reversible after initiation of non-invasive nocturnal ventilation, thus suggesting a causal link between ventilatory insufficiency and weight loss. In contrast, weight loss in the limb and bulbar onset groups was much less pronounced (mean $2.5 \pm 5.6\%$ and $6.0 \pm 5.1\%$, respectively). A few patients with limb onset (6 of 45) also lost 10% or more of their BMI in the early disease stages; in some cases this was due to other causes such as dieting, active colitis etc.

Conclusion: ALS cachexia is commonly observed in motor neuron disease with truncal or respiratory onset. Thus, it may be an indicator of hitherto undetected respiratory muscle involvement which may also in part explain the poor prognosis associated with this finding. Early severe weight loss in ALS patients without significant bulbar involvement should therefore prompt screening for ventilatory insufficiency.

P245 DECISION MAKING FOR GASTROSTOMY AND RESPIRATORY SUPPORT – VARIATIONS ACROSS UK HOSPICES

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Keywords: gastrostomy, non-invasive ventilation, hospices

Background: UK hospices are often involved in the care of people with ALS/MND. However over the last 10 years there has been increasing use of interventions of gastrostomy and non-invasive ventilation, and there is evidence that the use of these interventions varies across the country.

Objectives: The aim of the study was to ascertain the use of the interventions in several hospices and the attitudes of consultants in palliative medicine across the country to the use of these interventions.

Methods: An audit of notes of 60 patients who had died under the care of 6 hospices in the UK and Ireland allowed a comparison of the use of these interventions. A telephone audit of consultants in palliative medicine was undertaken, using a structured questionnaire, to ascertain their attitudes and their involvement in the decision making for the interventions.

Results: The audit of 60 patients showed variation from hospice to hospice: the use of percutaneous endoscopic gastrostomy (PEG) varied from 0% to 50% of patients, with a mean of 32%, and non-invasive ventilation varied for 10% to 50% with a mean of 18%. The questionnaire of over 20 consultants showed that they were rarely involved in the referral for either PEG insertion or non-invasive ventilation. They were concerned that the interventions could lead to distress to patients and families if they were used inappropriately and without clear discussion beforehand. There was need to provide clear and helpful information for patients and families and for the discussion to take place over a period of time, as a "process" rather than on a single occasion. They gave examples of the inappropriate use of interventions.

Discussion: Although UK hospices are often involved in the care of people with ALS/MND this may often be only for end of life care. There is limited involvement in the decision making for interventions that may promote quality of life and potentially extend life. These decisions may occur before hospice teams are involved and there are concerns that the information provided for patients and families may not always be adequate. The study shows that there may be a need for specialist palliative care teams to be working in a more collaborative way with neurology, rehabilitation and MND teams to facilitate decision making for the difficult decisions of interventions.

P246 "INSPIRATIONAL" – INSPIRATORY MUSCLE TRAINING IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: inspiratory muscle training, clinical trial, respiratory function

Background: Respiratory impairment, due to respiratory muscle weakness, is a major cause of morbidity and mortality in patients with amyotrophic lateral sclerosis-motor neuron disease (ALS-MND). Threshold loading may strengthen the inspiratory muscles and thereby improve patient prognosis.

Objectives: To determine whether a 12-week inspiratory muscle training (IMT) program would strengthen the inspiratory muscles and improve respiratory function in ALS-MND patients.

Methods: Nine patients were randomised to inspiratory muscle training and 10 to sham training. Primary endpoints were respiratory function (forced vital capacity, vital capacity), lung volumes and inspiratory muscle strength. Patients were assessed before, during and immediately after a 12-week training period, and at eight weeks follow-up.

Results: Although improvements in inspiratory muscle strength were observed in both treatment arms, there was a non-significant increase in maximum inspiratory pressure of $6.1 \pm 6.93\%$ in the experimental group compared to controls (95% confidence interval, $-8.58 - 20.79$; $P = 0.39$). The gains in inspiratory muscle strength were partially reversed during a period of training cessation. Similar trends were observed in respiratory function after the 12-week training period, with forced vital capacity $4.59 \pm 3.02\%$ (95% confidence interval, $-1.85 - 11.02$; $P = 0.15$) higher in the experimental group than control group. No serious adverse side effects were reported by patients.

Discussion and Conclusions: The present clinical trial is the first to evaluate the effects of IMT in ALS-MND patients in the context of a double-blind, randomised-controlled trial. Consistent trends for improvement were demonstrated across all respiratory parameters over multiple occasions. Measures of respiratory function suggested that IMT may have partially ameliorated the restrictive defect that develops in ALS-MND. In addition, measures of inspiratory muscle strength suggested that IMT was efficacious in strengthening the inspiratory muscles. As such, the results of the present trial would tend to support the hypothesis that despite an environment of ongoing denervation, the inspiratory muscles of ALS-MND patients are capable of responding favourably to a strength-training program.

P247 CAPNOGRAPHY AS A SCREENING TOOL FOR NOCTURNAL RESPIRATORY DISTRESS IN PATIENTS WITH ALS

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Keywords: capnography, pulse oxymetry, non-invasive ventilator

Background: Patients with amyotrophic lateral sclerosis (ALS) suffer from hypoventilation which can be worsened during sleep.

Objectives: To evaluate the efficacy of capnography monitoring for screening nocturnal hypoventilation in patients with ALS.

Methods: Thirty eight definite or probable ALS patients with subjective symptoms of hypoventilation or decreased vital capacity (<80% of normal) were included. Patients underwent nocturnal capnography and pulse oximetry monitoring, daytime arterial blood gas analysis (ABGA), measurement of forced vital capacity (FVC) and ALS functional score revised (ALSFRSr). Patients with subjective respiratory symptoms or decreased vital capacity were treated with non-invasive ventilation (NIV). Spearman's correlation test was used for statistical analysis.

Results: The degree of nocturnal hypercapnea was measured as duration of nocturnal hypoventilation, average level of $ETCO_2$, and maximal level of $ETCO_2$, using capnography. Those three values correlated well with degree of respiratory distress during sleep (scores to 'orthopnea' questionnaire in ALSFRSr; $r = -0.627 \sim -0.491$, $P = 0.004 \sim 0.033$) and compliance to NIV treatments ($r = 0.539 \sim 0.649$, $P = 0.001 \sim 0.012$). However the degree of nocturnal hypoxia, measured as duration of nocturnal hypoxia (defined as % of sleep when $SaO_2 < 95\%$ per total sleep), average nocturnal SaO_2, and minimal nocturnal SaO_2 had no significant correlation with nocturnal respiratory symptoms or compliance to NIV treatment.

Conclusion: Nocturnal capnography is a simple, efficient tool in screening hypoventilation and detecting respiratory symptoms in patients with ALS. Moreover, this can also be useful in predicting future compliance to NIV treatment.

P248 EARLY START OF NON INVASIVE MECHANICAL VENTILATION IN ALS PATIENTS

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Keywords: non invasive ventilation, disease severity, survival

Background: Many studies have shown improvement in survival for mechanically ventilated amyotrophic lateral sclerosis (ALS) patients. However, there is no consensus as to when non invasive ventilation (NIV) has to be started. One criterion is a vital capacity (VC) lower than 50% of predicted value.

Objectives: To compare the survival of NIV-treated ALS patients in early (VC >50%) vs late (VC <50%) disease stages.

Subject and Methods: Since 2004, 165 ALS patients (mean age: 63.7 ± 11.2 years) have been assisted. Thirty-three of 55 (33%) who died used NIV. Among these, only 24 patients,

with a diagnosis of ALS confirmed no longer than 12 months before, were studied. In particular, they were subdivided into two groups: twelve patients (mean age: 66.0 ± 9.1 years) who started NIV at a VC < 50% (mean VC: 35.5 ± 12.8%), and 12 (mean age: 58.5 ± 7.4 years) who began NIV with a VC > 50% (mean VC: 94.7 ± 23.3%).

Results: In the former group survival was 21.4 ± 12.2 months from diagnosis and 13.2 ± 8.0 months since using NIV; whereas in the latter group survival times were 18.7 ± 8.8 and 11.5 ± 7.2 months, respectively. Values did not differ significantly between groups.

Conclusions: These data show that in early NIV-treated ALS patients severity of respiratory impairment does not influence survival.

P249 EFFECT OF A MULTIDISCIPLINARY ALS CLINIC ON NIV TOLERANCE AND SURVIVAL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND BULBAR IMPAIRMENT: A PROSPECTIVE STUDY

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Keywords: non-invasive ventilation, survival, bulbar impairment

Background: The effect of non-invasive ventilation (NIV) in prolonging survival and improving quality of life in ALS patients with respiratory failure (RF) has been largely demonstrated, but it is currently under-prescribed. One restricting factor to the efficacy of NIV is bulbar involvement, but little is known about predictors of NIV tolerance.

Objectives: To evaluate the factors associated with NIV tolerance and survival in ALS patients.

Methods: All consecutive ALS patients who came to the Center between January 2001 and December 2006 were prospectively monitored with RF and indication to NIV. NIV was offered according to current guidelines. Patients were assessed by: demographical and clinical characteristics, pulmonary function and disability (as measured by ALS Functional Rating Scale (ALS FRS)). After NIV indication, subjects were followed-up in the ALS Clinic regularly at three-month intervals. According to current accepted criteria, the tolerance to NIV was defined as the ability to use the ventilator for more than 4 consecutive hours/day.

Results: A total number of 115 ALS patients (75 males and 40 females) with indication to NIV were enrolled. Among them, 33 patients had a bulbar disease onset, but 38 had developed severe and 65 mild/moderate bulbar impairment at NIV initiation. Sixty patients (52.2%) were tolerant to NIV. As expected, the majority of the intolerant patients had mild/moderate (47.3%) or severe (43.63%) bulbar impairment at NIV initiation. Survival analysis (total survival and survival from the time when NIV was indicated) demonstrated a longer survival in the tolerant group ($P < 0.001$). Interestingly, among the group with severe bulbar impairment, patients who tolerated NIV survived longer than those who were intolerant ($P < 0.001$). Further, we found that the bulbar patients tolerant to NIV come to the ALS Clinic more often than those intolerant after NIV indication ($P = 0.0001$).

Conclusions: This study shows that a regular follow-up in a multidisciplinary ALS Clinic after NIV indication could increase tolerance to NIV and survival, even in patients with severe bulbar impairment. The effect of clinical type must be considered in management of respiratory failure.

P250 FACTORS ASSOCIATED WITH NON-INVASIVE POSITIVE PRESSURE VENTILATION COMPLIANCE IN ALS/MND PATIENTS

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Keywords: non-invasive positive pressure ventilation, compliance, BIPAP

Background: Despite Class I evidence that non-invasive positive pressure ventilation (NIPPV) improves both survival and quality of life (QOL), compliance with the intervention remains low.

Objectives: To prospectively examine factors which influence NIPPV compliance.

Methods: Seventy-three ALS patients with a forced vital capacity (FVC) > 50% were enrolled in the multi-center pilot study of Early Nutrition and NIPPV. Patients with a FVC over 80% at baseline were initiated on NIPPV when their FVC was 75–85% (Group1 – Early intervention). Patients with a FVC between 50–80% were started when their FVC was 45–55% (Group 2 – Standard of Care). Patients were educated about NIPPV prior to initiation. Respiratory therapist visits were made three times the first week, twice the second and once in the third and fourth weeks with monthly visits during the rest of the study. NIPPV compliance was defined as > 4 hours of use on 60% of days based on computer downloads 4 weeks after initiation of treatment. Patients were followed over 12 months and were asked to complete a 17 item "symptom score" after initiating NIPPV. The instrument asked patients: "While using NIPPV, how often do you experience the following symptoms?" and patients completed a scale from 0 (never) to 10 (all the time) for each symptom.

Results: Of the 73 participants in the NIPPV arm of the study, 57 subjects were offered NIPPV (36 in Group 1 and in 21 in Group 2). Objective data from downloads were available from 47 of the 57 participants offered NIPPV (30/36 Group 1 and 17/21 Group 2). By day 28 (week 4) after initiation of NIPPV, the compliance rate was 53.3% for Group 1 and

70.6% for Group 2. For the non-compliant patients in both groups, the most frequent symptoms included: excessive dryness of the nose or throat passages (mean score 3.67), mask discomfort (3.28), air leakage from the mask (3.11), waking up frequently during the night (2.78), a sense of suffocation or claustrophobia (2.39), and soreness in the nose or throat passages (1.78). The remainder of symptoms did not appear to be related to non-compliance: running nose, headaches, ear pain, marks or rash on face, complaints from partner about noise from the machine, or bloating. Interestingly, when this non-compliant group was asked, "Do you believe there has been an improvement on your QOL?", 72% responded "yes".

Discussion and Conclusion: The majority of symptoms reported by patients within the first 4 weeks of initiating NIPPV are related to issues that are potentially resolvable with aggressive respiratory therapy intervention. Ensuring proper humidification and finding an interface that is comfortable and seals properly are imperative to improving compliance.
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P251 NON-INVASIVE POSITIVE PRESSURE VENTILATION IN AMYOTROPHIC LATERAL SCLEROSIS: PREVALENCE, APPROACH AND BARRIERS TO USE AT CANADIAN ALS CENTRES

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Keywords: NIPPV, prevalence, initiation

Background: Non-invasive positive pressure ventilation (NIPPV) has become the standard of care in the initial management of respiratory insufficiency in ALS (1,2). Nonetheless, considerable variability in the prevalence of NIPPV use has been reported in the European and U.S. ALS patient populations (3).

Objectives: To evaluate: 1) the prevalence of NIPPV and invasive mechanical ventilation via tracheostomy (TV) therapy, 2) the approach to NIPPV use, focusing upon the currently employed initiation criteria and 3) the barriers influencing NIPPV utilization in Canada.

Methods: A descriptive survey research design aimed to acquire quantitative data and open-ended responses from an active physician at each of the 15 multidisciplinary Canadian ALS centres.

Results: The principal findings were: 1) NIPPV and TV therapy are used in 18.3% and 1.5% of Canadian ALS centre patients, respectively. 2) Symptoms of respiratory insufficiency, namely orthopnea (clinical importance rated at 9.00/10 $\pm$1.48, mean $\pm$SD), dyspnea (8.27 $\pm$1.95) and morning headache (7.55 $\pm$1.21) are the most significant indicators for NIPPV initiation. 3) Secondary to symptoms, nocturnal oximetry, then forced vital capacity (FVC) and morning blood gases, are the investigations most important to NIPPV initiation. 4) The primary barriers to NIPPV utilization are patient intolerance (70% of centres) and lack of access to respirologists and/or ventilation technologists (50% of centres).

Discussion: These data may reflect an international trend towards an increased use of NIPPV therapy and a concurrent restriction of TV application in ALS patient care, which for some represents a translation of evidence into clinical practice. In addition, the results provide support for the primary importance of symptom assessment in the decision to institute NIPPV therapy. Subsequently, greater weight is being placed on investigations that may facilitate an earlier implementation of NIPPV (i.e. nocturnal oximetry over FVC). Nevertheless, similar to previous international analyses, inter-centre variability persists with respect to NIPPV utilization (3).

Conclusions: As this variability has an impact upon the management of ALS patients, future studies should focus upon: the continued establishment of more definitive NIPPV initiation criteria, with an emphasis upon respiratory symptoms; and the attenuation of barriers to NIPPV use, so as to ensure optimal care for all ALS patients.

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P252 VENTILATORY SUPPORT IN ALS: TIMING, CHOICE AND OUTCOME IN A MULTI-ETHNIC POPULATION

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Keywords: ventilatory support, BiPAP, multi-ethnic

Background: Amyotrophic lateral sclerosis (ALS) frequently causes death within 5 years of onset. Almost all deaths in ALS are due to pulmonary complications resulting from respiratory muscle weakness and bulbar involvement. Timely ventilatory support can reduce the work of breathing and dyspnea, can improve gas exchange, improve quality of life and it can prolong survival in patients with ALS. The timing, choice and outcome of ventilatory support in a cross-ethnic population of ALS remain unexplored.

Objectives: To study the timing, choice and outcome of ventilatory support in an ethnically diverse ALS population.

Methods: The results of ventilatory use was retrospectively analyzed in an ethnically diverse group of 205 patients who were seen and followed up at the Kessenich Family MDA-ALS Center from 2002 through 2007 and in whom clinical records were available from diagnosis to death.

Results: The cohort of 205 patients (male 122, female 83; mean age at death 64.9 years (yr), range 34–91 yr) comprised of 128 White Caucasians (W, 62%), 61 Hispanics (H, 30%), 12 African Americans (AA, 6%), and 4 Asians (A, 2%). 139 patients (68%) elected to receive BiPAP (W 60.2%, H 80.3%, AA 83.3%, A 75%). In BiPAP group, 93 patients (67%) had limb-onset and 46 patients (33%) had bulbar-onset ALS.

Acceptance of BiPAP showed a steady increase in all groups during the study period (61% in 2002, 72% in 2007). Survival from the onset of the disease was 41.2±26 months (mo) (mean and SD; BiPAP group 45.1±28.6 mo, non-BiPAP group 36.7±22.7 mo). 11 patients (5.4%) opted for invasive ventilation (W 4%, H 8%, AA 8%, A 0%). 66 patients (32.3%) received a feeding tube before or soon after ventilatory assistance (W 26%, H 41%, AA 50%, A 50%). 102 patients (50%) died in the hospice program (W 48%, H56%, AA 58%, A 0%). Age of onset, gender, site of disease-onset and mean survival were comparable across the ethnic groups. Tolerance and quality of life following ventilatory assistance was generally good in all groups of patients.

Discussion and Conclusions: Acceptance of non-invasive ventilation showed a steady increase during the study period and BiPAP significantly prolonged survival in all ethnic groups. The findings of relative under utilization of BiPAP ventilatory support among ethnic groups require prospective studies to determine the underling cause(s). Patients with ALS rarely elect invasive ventilation.

P253 COST-EFFECTIVENESS OF HOME TELEMONITORING NON-INVASIVE VENTILATION IN ALS PATIENTS

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Keywords: cost-effectiveness, telemonitoring, wireless solution

Background: A prospective trial of home-ventilated ALS patients was recently conducted documenting less healthcare utilization and a trend towards better quality of life and survival outcomes. Nevertheless, no study has yet evaluated healthcare costs associated with telemetry for assessing compliance to non-invasive ventilation (NIV). Home telemonitoring of NIV by modem communication may substantially cause a significant impact on total cost of care.

Objectives: To analyse direct (hospital and NHS) and indirect (patient and caregiver) costs of following-up NIV compliance via modem.

Methods: A prospective controlled trial of consecutive ALS patients, randomly assigned according to their residence area to G1 (patients nearby hospital; office-based follow-up) and G2 (patients outside hospital area; telemetry device based follow-up) was performed. Total NHS direct cost of care was determined by summing medical costs related to General District Hospital (GDH)-based outpatients visits and hospitalizations. Hospital direct costs included transportation to/from hospital, office visit per hour cost and daily maintenance of equipment (with/without modem). Non-medical costs were considered as days of wage lost due to caregiver absenteeism. Annual costs analysis with all variables adjusted to the survival period with NIV in days was performed.

Results: G1 included 20 patients aged 60±10 (14 males, 16 spinal-onset and 4 bulbar-onset) and G2 included 19 patients aged 62±13 (13 males, 11 spinal and 9 bulbar-onset forms). No statistically significant differences were found among the two groups regarding clinical and demographic characteristics at admission. (i) NHS costs evaluation showed a 55% reduction on average total costs (G1: 19,665±23,507€ vs G2: 8,909±4,619€; $P = 0.05$) with a statistically significant decrease of 81% on annual costs (G1: 44,134±50,607€ vs G2: 8,186±6,553€; $P = 0.005$) in G2. (ii) Hospital costs were found to be significantly higher in G2 regarding to the total costs (64% average increase, $P = 0.008$) but not annual costs (7% average increase, $P = 0.36$). (iii) No statistical difference was found concerning caregiver expenses from abseentism due to office visits or hospitalizations ($P = 0.15$).

Conclusion: At the expense of an initial financial constraint to the hospital per year (non-significant), this telemonitoring instrument for NIV compliance in ALS patients has proved to be cost-effective along with survival with NIV, thereby representing major cost savings to the NHS in the order of €700,000 per year.

P254 DESCRIPTIVE STUDY OF A HIGH RESOLUTION NEURO-RESPIRATORY UNIT

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Keywords: non-invasive ventilation, multidisciplinary, tracheostomy

Background: Patients with Amyotrophic Lateral Sclerosis (ALS) need multidisciplinary care. The development of Multidisciplinary Units may develop and provide better assistance to patients. The aim of this study is to evaluate the results of the early respiratory intervention during the first two years of an ALS Unit.

Methods: Observational study of the patients attended in the ALS Unit during the period from March 2006 to December 2007. The following information has been recorded: age, gender, ALS initial form (bulbar or spinal), months of evolution up to non-invasive ventilation (NIV), adjustment to NIV, type of ventilator, tolerance to the ventilation and tracheostomies.

Results: 45 patients diagnosed with ALS were checked, 24 men and 21 women, mean age of 65.88 years old (ranging from 41 to 91 years old). The mortality was 37.8%. 3 patients were lost in the follow-up. According to the initial form of ALS, 30 patients were spinal (66.7%) and 14 bulbar (31%), with a major mortality in the spinal group (46%) vs bulbar (21.42%). 20 patients were adapted to NIV (44.4%), with a major use of the volumetric ventilators (60%), than pressure ventilators (40%), leaving BIPAP for the patients who needed an orofacial mask. 53.33% of spinal ALS were adapted to NIV (37.5% to BIPAP and 62.5% to volumetric ventilator) and 28.57% of bulbar ALS to NIV (50% to BIPAP and 50% to volumetric ventilator). The evolution of the disease up to the start of using NIV was variable (35.88 months of average), which shows the variability of the disease. The tolerance to NIV was good at 66.7% of cases. Tracheostomies were performed on 5 patients (4 spinal and 1 bulbar).

Conclusions: During the first two years of the ALS Unit, the spinal ALS group was more numerous than the bulbar group, with a major adaptation to NIV. Volumetric ventilators were used more frequently and its tolerance was good. Tracheostomy was practised in more spinal than in bulbar patients.

P255 EFFECTS OF NIPPV INITIATION ON PULMONARY FUNCTION TESTING IN ALS SUBJECTS

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Keywords: NIPPV, FVC, respiratory failure

Background: Recent literature indicates a significant effect of non-invasive positive pressure ventilation (NIPPV) both on survival and quality of life in ALS patients who are compliant (NIPPV >four hours daily) leading to the present recommendation that NIPPV should be initiated when ALS patients are symptomatic and/or when the FVC declines to 50% of expected. While some studies have demonstrated a slowing of the rate of decline in FVC accompanying the survival benefit, others have demonstrated an initial deterioration in the FVC following the start of NIPPV leading to the concern that NIPPV may actually weaken respiratory muscles initially.

Objectives: To examine the early effects of NIPPV on respiratory function in compliant and non-compliant patients.

Methods: Seventy-three ALS patients with a forced vital capacity (FVC) >50% were enrolled in a multi-center pilot study of Early Nutrition and NIPPV. Patients with a FVC over 80% at baseline were initiated on NIPPV when their FVC was 75–85% (Group 1 – Early intervention). Patients with a FVC between 50–80% were started when their FVC was 45–55% (Group 2 – Standard of Care). NIPPV compliance was defined as consistent use of at least >4 hours per night based on machine downloads. Pulmonary functions (sitting and laying FVC, nasal inspiratory pressure (SNIP) and maximum voluntary ventilation (MVV), MIP and MEP) were measured before and 4–6 weeks after the initiation of NIPPV.

Results: Of the 73 participants in the NIPPV arm of the study, 57 subjects were offered NIPPV (36 in Group 1, 21 in Group 2). Objective data from downloads were available from 47 of the 57 participants offered NIPPV. The compliance rate was 53.3% for Group 1 and 70.6% for Group 2. FVC in compliant subjects dropped less then noncompliant subjects over the first two months ($71.8 \pm 1.4\%$ to $68.6 \pm 18.3\%$ vs $70.9 \pm 14.9\%$ to $63.5 \pm 19.7\%$ predicted) although it did not reach statistical significance. Similarly, the MVV declined less in compliant subjects (58.4 ± 34.5 L/min to 56.7 ± 38.3 L/min) compared to noncompliant subjects (52.9 ± 28.5 to 44.1 ± 26.5 L/min). The 7.4% drop in FVC between pre and post NIPPV in noncompliant subjects was statistically significant ($P = 0.003$) while the drop of 3.2% in compliant subjects was not. Similarly, the decrease in MVV was significant in noncompliant subjects (8.8 ± 10.5, $P = 0.003$), but not in compliant (3.9 ± 14.6). There was no immediate drop in FVC observed.

Discussion and Conclusions: There was less decline of both FVC and MVV in NIPPV compliant compared to noncompliant subjects observed within the 4–6 weeks following the initiation of NIPPV although the difference was not statistically significant. The drop of FVC between pre and post-NIPPV in compliant subjects was not significant and contrasts previous reports of a more rapid decline initially. This suggests that there is no initial weakening of the respiratory muscles.

P256 THE USE OF NON-INVASIVE VENTILATION DURING PERCUTANEOUS ENDOSCOPIC GASTROSTOMY INSERTION IN PATIENTS WITH IMPAIRED RESPIRATORY FUNCTION

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Keywords: non-invasive ventilation, PEG, RIG

Background: Percutaneous endoscopic gastrostomy (PEG) is the standard management of bulbar ALS patients at risk of malnutrition and aspiration. PEG is not recommended in patients with significantly impaired respiratory muscle strength (RMS) determined by a vital capacity (VC) below 50% predicted and/or a sniff nasal inspiratory pressure (SNP) below 40 cm water, although VC and SNP may underestimate RMS in bulbar ALS patients. Radiologically inserted gastrostomy (RIG) is well tolerated in patients with impaired RMS. However, RIG is not available throughout the United Kingdom. This necessitated the centre to develop a novel approach of performing PEG with non-invasive ventilation (NIV) available to be delivered before, during or after the procedure, if required.

Objectives: 1) To determine if the use of NIV permitted the insertion of PEG in bulbar ALS patients with significantly impaired RMS. 2) To determine if the above criteria were underestimating the patients RMS and if it was feasible to adopt less stringent threshold values to determine fitness for PEG.

Methods: The cases of patients referred for a respiratory muscle assessment were reviewed to determine fitness for PEG insertion over a 53-month period. Patients had VC, SNP, capillary blood gases and overnight pulse oximetry. 24 patients were identified that had inadequate respiratory function for PEG insertion as determined by the above criteria or already being established on NIV. These patients had a PEG insertion under sedation with the facilities and staff to provide NIV during the procedure or at recovery should it be required if the patient desaturated or developed respiratory compromise. If NIV was required during the procedure, the complications of PEG insertion and the date of death were determined.

Results: There was one death in the 24 patients within 30 days with all other patients surviving beyond this period following PEG insertion.
Eleven patients were already established on NIV. All required NIV during the procedure. PEG was successfully inserted in all but one patient who had significant desaturation and required a second attempt, which was successful.
Thirteen patients were not established on NIV. Three patients declined to have PEG under NIV cover, preferring to use local hospitals. None of the patients declining our service had complications during the procedure, but all patients had difficulty with respiratory tests and therefore their RMS may have been underestimated. Of the remaining 10 patients, 5

required NIV during the procedure. One patient required a second attempt for successful PEG placement and a PEG was unable to be placed in one patient due to a high stomach.

Conclusions: PEG with precautionary NIV available is a safe alternative procedure to RIG in patients with impaired RMS. PEG can be placed in patients currently using NIV.

P257 THE STUDY OF NIPPV USING FULL-FACE MASK REMODELED TO SUPPORT ENDOSCOPE IN PER-ENDOSCOPIC GASTROSTOMY IN ALS PATIENTS

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Keywords: remodeled mask, NPPV, PEG

Background: ALS treatment guidelines recommend performing PEG while the patient's%VC is greater than 50%. But sometimes PEG is required in patients already have respiratory dysfunction. In this case, the risk of worsening respiratory function during PEG is much higher. In 2005 the usefulness of NIPPV using a remodeled full-face mask to support endoscope during PEG was reported. We started to use this method in 2006.

Objectives: To evaluate usefulness of NPPV using remodeled full-face mask to support endoscope with larger number of patients.

Methods: Thirty-seven consecutive PEGs that were performed in ALS patients (14 men, 23 women) since March 2006 to February 2009 were studied. Seventeen patients were already on NIPPV (9 cases night-time only, 8 cases all day). Blood gas analysis, sniff nasal inspiratory pressure measurement (SNIP) and overnight SpO_2 monitoring were performed other than %VC prior to PEG. The condition of the patient, vital signs and SpO_2 were analyzed during PEG. PEG was performed using direct methods. Tentative criteria were used for indication of NIPPV to support endoscope as one of following 1) %VC less than 50%, 2) PCO_2 greater than 50 mmHg; 3) SpO_2 less than 88% lasts for more than 5 minutes during sleep. 4) Rapid decrease of SNIP. 5) Dyspnea. Pethidine Hydrochrolide and Midazolam injection were used as pre-operative medication.

Results: Only 11 cases had a%VC greater than 50% while 13 cases with $30\% < \%VC < 50\%$, 4 cases with $< 30\%$, 2 cases were immeasurable. Only 22 cases have $PaCO_2$ less than 45 mmHg while 9 cases with 45 mmhg $< PaCO_2 < 50$ mmHG, 6 cases with > 50 mmHg. Based on inclusion criteria, In 27 cases PEG with NPPV using remodeled full-face mask to support endoscope were performed. Despite two-thirds of patients having%VC less than 50%, PEG was safely performed in 26 out of 27 patients using NPPV using full-face mask remodeled to support endoscope. SPO_2 was temporally decreased in 12 patients but recovered with oxygen therapy. Only one patient had aspiration during preparation procedure and PEG was cancelled.

Discussion and Conclusions: PEG was safely performed even in ALS patients with respiratory dysfunction with NIPPV using full-face mask remodeled to support endoscope. Thus this method is very useful for patients that already have decreased respiratory function.

P258 STUDY RESULTS OF DIAPHRAGM PACING IN EXTREMELY LOW FORCED VITAL CAPACITY PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS/MOTOR NEURON DISEASE: IS THERE A ROLE AT END STAGE ALS?

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Keywords: diaphragm, diaphragm pacing, respiration

Background: Trials of diaphragm pacing (DPS) in ALS/MND had been limited to patients with FVC above 45% predicted at surgical implantation due to concerns of inability to extubate or tolerance of surgery. There is also concern of insufficient intact lower motor neurons with end stage ALS/MND and that remaining motor units are inadequate for pacing to have any effect on respiration.

Objectives: To assess the safety and efficacy of DPS in subjects with FVC below 45% predicted.

Methods: Prospective, nonrandomized, controlled, interventional feasibility trial assessing safety and efficacy through patient assessment of breathlessness, electromyography of diaphragm function and nocturnal oximetry with and without DPS.

Results: Subjects were enrolled between September 2007 and March 2009 with an average FVC at implantation of 28% predicted (range 14% to 36%). Subject 1 is still alive 19 months post implantation with benefits shown from increased diaphragm burst EMG activity, DPS overcoming nocturnal hypoventilation and Subject 1 is still not using non-invasive ventilation (NIV). Subject 2 survived 9 months post implantation and with benefits shown by increase in diaphragm EMG and decreased initial use of NIV. Subject 3 was initially extubated but rapidly tired out requiring re-intubation with subsequent withdrawal of life support. The patient had extreme malnourishment (BMI of 14) with significant weight loss awaiting DPS and gastrostomy placement. Although he had a stimulatable diaphragm at surgery, no benefit to DPS can be claimed. Subject 4 survived 10 weeks post implantation before withdrawing support and expiring. Surgical evaluation showed a significant loss of diaphragm motor units. Although survival was only 10 weeks patient benefits were seen by increased diaphragm burst activity during EMG, improved breathlessness scores, dependence on DPS to maintain night oxygenation and the need to turn off DPS to allow death. Subject 5 was able to be safely implanted and had a large amount of upper motor neuron involvement of the diaphragm manifested in substantial post-implantation tidal volumes and diaphragm movement under stimulation. While awaiting regulatory approval for implantation the patient had entered hospice, but decided to leave hospice to have the implantation. Within a week of implantation she decided to once again withdraw all life support and expired. Although DPS had the potential to improve ventilation, no beneficial outcome can be claimed for Subject 5 due to her decision to withdraw support.

Conclusion: DPS benefits can be seen in selected low FVC ALS/MND subjects. The societal expense of a surgical procedure has to be addressed with patient wishes preoperatively to prevent unnecessary expenses to the health care system. Patients must understand that DPS is only a

symptomatic treatment of diaphragm respiration. Presently, we only implant motivated patients with documented intact stimulatable diaphragm motor units through phrenic nerve conduction studies with diaphragm movement assessment.

P259 RESPIRATION AND AMYOTROPHIC LATERAL SCLEROSIS/MOTOR NEURON DISEASE: THE ROLE OF THE DIAPHRAGM AFTER A DECADE OF EXPERIENCE WITH DIAPHRAGM PACING

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Keywords: diaphragm, diaphragm pacing, respiration

Background: Diaphragm pacing (DPS) has been used in a series of trials to help respiration ranging from spinal cord injured (SCI) patients with pure upper motor neuron (UMN) loss of control to patients with pure lower motor neuron (LMN) loss from phrenic nerve transection along with direct analysis of stimulation of normal subject diaphragms. ALS/MND involves both UMN and LMN involvement of the diaphragm.

Objectives: To describe how ALS/MND diaphragm pathophysiology can affect respiration.

Methods: Prospective, nonrandomized, controlled, interventional trials under IRB and/or FDA approval for use of DPS in human subjects at a single institution. Comparisons of diaphragm function from normal to different diseases states were compared to ALS/MND.

Results: From 1999 to 2009 over 128 human diaphragms were surgically evaluated for motor point stimulation for ventilation with over 250 patient-years of DPS use. Subjects ranged from 12 weeks to 77 years old. Diaphragm evaluations included normal subjects undergoing elective laparoscopy to SCI patients on ventilators over 25 years. ALS/MND diaphragms evaluated varied from FVC of 89% predicted to continuous mechanical ventilation with tracheostomy for 18 months. Pre-operative pulmonary function tests do not predict surgical stimulation results. Patients can have high FVC but poor diaphragm function or low FVC and excellent stimulatable diaphragms. ALS/MND involvement of the diaphragm is heterogeneous. It can be visualized as radial bands or regions of loss of motor units and can functionally involve each hemi-diaphragm differently. Using frequency stimulation to differentiate muscle fiber type, ALS/MND diaphragms that had primarily UMN involvement or suppression of diaphragm function with NIV demonstrated conversion to fast twitch (type IIb) muscle fibers. DPS can convert ALS/MND diaphragms to more functional type I muscle fibers. Used in conjunction with NIV, DPS can prevent atrophy and conversion. Implanted DPS electrodes can be used to assess diaphragm burst activity which appears to correlate with strength of diaphragm contraction and can be serially followed. This has been utilized to identify and treat central sleep apnea in ALS/MND. ALS/MND patients with primary LMN involvement have diffusely thin non-stimulatable diaphragms. DPS increases respiratory compliance by 17% which decreases the work of breathing. ALS/MND patients may develop daytime hypoventilation leading to hypercarbia which DPS can overcome. During disease course use of DPS can go from several 30 minutes conditioning sessions a day to 24 hours continuous use to maintain ventilation with the need to turn off DPS if death is sought. In tracheostomy mechanical ventilated patients, some with intact phrenic motor neurons remaining, DPS decreases peak airway pressure and improves posterior lobe ventilation leading to less atelectasis and risk for pneumonia.

Conclusion: ALS/MND patients with respiratory complaints and intact phrenic motor neurons that the patient cannot control due to UMN involvement are candidates to improve diaphragm movement and ventilation with DPS.

P260 HYPOACUSIS IN ALS PATIENTS RECEIVING LONG-TERM VENTILATION

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Keywords: hypoacusis, long-term ventilation, decision-making

Background: ALS is defined as a degenerative disease that selectively affects motor neurons. In a subgroup of ALS there is evidence for non-motor involvement such as cachexia, ophthalmoplegia and autonomic impairments. Hypoacusis, however, has not been reported in ALS.

Methods: Three ALS patients, patient 1(P1) (32 year old female), patient 2 (P2) (47 year old female) and patient 3 (P3) (48 year old male) were retrospectively studied, who underwent tracheotomy after 35, 26 and 25 months of disease, respectively. All received continuous ventilation. 78, 33 and 31 months into the course of the disease these patients developed bilateral hearing impairment.

Results: Two cases (P1 and P2) were diagnosed as hypoacusis based on sensorineural hearing loss as demonstrated by audiometry (P1) and auditory evoked potentials (P2). A structural lesion in the middle ear was clinically and pathoanatomically excluded. In the third patient (P3), conductive hearing loss was identified, caused by chronic serous tubotympanal catarrh.

Discussion: Sensorineural hearing loss in the context of ALS is etiologically unknown. The reported cases (P1 and P2) suggest a neurodegenerative process involving the auditory system. By contrast, conductive hearing loss is presumably caused by bulbar symptoms, including paralysis of the tensor and levator veli palatini muscles. These symptoms disturb the equalization of pressure in the middle ear and can result in chronic serous tubotympanal catarrh.

Conclusion: Through altering the natural course of the disease, the administration of long-term ventilation leads to previously undiscovered aspects in patients and subsequently complicates patient management. Given the increasing use of long-term ventilation, atypical symptoms are of growing clinical relevance. Identifying hypoacusis is critically important in complex decision-making and particularly in the adaptation of electronic communication systems, in the withdrawal of therapy or palliative care.

P261 WITHDRAWAL OF INVASIVE HOME MECHANICAL VENTILATION IN ADVANCE STAGE ALS PATIENTS

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Keywords: withdrawal, invasive ventilation, advance stage ALS

Background: Due to the growing use of artificial respiration in amyotrophic lateral sclerosis (ALS), physicians are increasingly confronted with patients seeking discontinuation of therapy. Yet there are few systematic investigations of the withdrawal of invasive home mechanical ventilation (IHMV). IHMV in ALS varies greatly between centres and countries, depending on the physicians' attitudes. One of the controversial aspects of this procedure is the issue of life support withdrawal. According to Danish legislation patients must not only give informed consent to any therapy; they can also ask to have therapy terminated at a time when life has lost its meaning according to their own definitions.

Objectives: To describe and discuss the medical aspects of terminating IHMV in patients with advance stage ALS.

Methods: A retrospective, descriptive analysis was made of all 12 ALS-patients (4 females) median age 61 years (range 39–69 years) cared for at our centre from 2002 to 2009 and who decided to withdraw their consent of IHMV. All patients were on 24 hour IHMV. The time from initiation of IHMV to decision to terminate were recorded. The reasons for the request and the circumstances of the procedure of termination were obtained.

Results: In all cases withdrawal of treatment was discussed with the patient before the initiation of IHMV. All patients decided that treatment should be withdrawn should they develop a locked in state.
The median time from initiation of IHMV to decision of termination was 21.5 months (range 0.23–35 months). The reason for requests was for all patients a general loss of meaning in life, but in three patients additional factors were: recurrent infections, inability to speak and bleeding from colorectal cancer. In one case withdrawal was performed after the patient developed a locked in state. The final decision of withdrawal was taken after a median of 2 conversations (range 1–3) with median 10 days (range 1–87 days) interval to ensure the durability of the decision. According to the patients' wishes the procedure took place in the home in 8 cases and at the hospital in 4 cases. Deep sedation was achieved with high dose Morphine (median 100mg, range 50–460 mg), Diazepam (median 100 mg, range 20–260 mg), before disconnecting the ventilator. Median time from discontinuation to apnoea was 15 minutes (range 0–1080 min) and to death 30 minutes (range 5–1080 min). An exemplary case will be presented.

Conclusions: A majority of the patients decided that treatment should be withdrawn before they developed a locked in state. The reasons for the request for withdrawal of treatment were a general loss of meaning in life. Withdrawal of IHMV after deep sedation may be a medically, legally and for the patient justified procedure to ensure a peaceful death in patients with advanced stage ALS and 24 hour IHMV.

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THEME 11 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P262 USAGE OF CUSTOMIZED WHEELCHAIRS BY PATIENTS WITH ALS

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Keywords: wheelchairs, cost, falls

Background: Wheelchair issues in ALS have rarely been studied (1). Customized wheelchairs are commonly prescribed for patients with ALS. In our clinic, 51% of patients who were seen for the first time during the year 2006 have used or are currently using customized wheelchairs. Wheelchairs maintain mobility once increased fall risk makes independent ambulation unsafe. They provide independence, comfort and pressure relief, allow increased participation in activities of daily living and reduce energy expenditure. Obtaining a customised wheelchair can take months and cost tens of thousands of dollars. Practice guidelines are not available to determine the optimal time to recommend a customized wheelchair. Our patients often wait an excessive amount of time, often past the point of need, for their customised wheelchairs. Some use the chairs for too short a time to justify the wait and cost.

Objectives: To understand the use of time and resources in obtaining and using customized wheelchairs in our patient population and to generate research questions for future study.

Method: Under a CUMC IRB approval, we reviewed the charts of patients with ALS who were evaluated for a customised wheelchair from April 2008–April 2009. This time period was chosen to coincide with the initiation of performing wheelchair evaluations within our multidisciplinary clinic. Wheelchairs were recommended to those who had a history of falls, demonstrated gait deficits and could no longer walk safely.

Results: A total of 38 patients' charts were reviewed. 52% of patients were male and mean age was 66 years old. The mean ALSFRS and FVC (% normal) were 35 and 81% at diagnosis and 32 and 66% at time of wheelchair recommendation. The mean number of months from diagnosis to recommendation of a wheelchair was 30. Patients waited a median of 4 months (range 2–31 months). The average cost of each wheelchair was $25,648.97. Of the 38 patients, 24 fell and 13 were delayed in starting hospice while waiting for the delivery of their wheelchairs. Of the 38 patients, 8 have died. 4 of the 8 never received their wheelchairs prior to their death. The other 4 utilized their wheelchairs for a mean of 3 months prior to death (range 1–5 months).

Discussion and Conclusion: Streamlining and expediting the process of obtaining customized wheelchairs for ALS patients may allow maximum utilization of these devices improving the cost/benefit ratio. This may reduce falls and allow for quicker entry into hospice services. A prospective study investigating quality of life and medical economics is essential.

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Reference:

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P263 POWER WHEELCHAIR PRESCRIPTION, UTILIZATION, SATISFACTION AND COST FOR PATIENTS WITH ALS/MND; PRELIMINARY DATA FOR EVIDENCE BASED GUIDELINES

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Keywords: assistive technology, neurorehabilitation, durable medical equipment

Background: Rapid disease progression in ALS/MND patients leads to unique needs for power wheelchair selection and continuous requirements for power chair updates to meet functional changes. There are no evidence-based guidelines available for employment of durable medical equipment specifically in the ALS/MND population. Current management of mobility equipment for ALS/MND patients is primarily based on the experience of clinicians and feedback from patients.

Objectives: To determine the features that ALS/MND patients require and choose on their power wheelchairs, how often the chairs/features are used and whether people like those choices over time.

Method: A questionnaire was sent to a convenience sample of 45 ALS/MND patients who are power wheelchair users and are currently seen at our multidisciplinary ALS/MDA clinic; 27 males and 18 females, with a mean age of 57.9 years (27–85). The average total ALSFRS-R score was 16 (0–38), and 17/45 of the patients used ventilators. The average time from first symptom to time of chair was 36.08 months (16–75), and from chair to questionnaire was 28.17 months (1–71). Thirty questionnaires (67%) were returned.

Results: Ninety-three percent (28/30) of respondents received their evaluations at a multidisciplinary ALS clinic, one via the Department of Veterans Affairs, and one was unknown. Seventy percent (21/30) of patients thought the chair evaluation was timed correctly, and 20% (6/30) wished they had started sooner. Forty-three percent (13/30) of people were able to walk a few steps, and 57% (17/30) were able to stand when their chairs arrived. We compared how many

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times a day they used tilt, recline, power legs, and seat elevate after receiving the chair and currently as well as overall time spent in their chairs for the same periods. When they first received the chair, 78% (22/28) were satisfied with the overall comfort of the chair, and 86% (23/27) were satisfied with the ease of use; currently, 66% (18/27) are satisfied with the overall comfort, and 66% (18/27) are satisfied with ease of use. The average cost for the power chairs was $26,404 ($19,376–$34,311), and the average cost per month is $937 ($242–$2,450). Overall, 88% (26/30) of respondents said they would get the same type of chair with the same features again and 81% (21/26) felt that the chair was good value for the cost.

Conclusions: We obtained first hand knowledge from 30 ALS/MND patients who are current power wheelchair users, on their utilization and satisfaction with their power wheelchairs from initial to current use. Information reported may assist wheelchair evaluators with what ALS/MND patients need and want, and will direct clients toward choices that will suit them for the long term. Based on this survey, ALS/MND patients are overall satisfied with their chairs, and further study of timing, adjustability, and customization of the equipment will help with the analysis of the cost/benefit to these patients.

P264 PILOT STUDY OF SUPPORTED TREADMILL AMBULATION TRAINING FOR AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: physical exercise, gait, active assisted exercise

Background: Repetitive rhythmic physical exercise (RRPE) has a positive health outcome in the general population. The effect of exercise in ALS is controversial. Exercise parameters such as type, mode, and dosing (duration, frequency and intensity) are lacking for ALS. Ambulatory patients with assistive devices (AD) can not engage in RRPE without assistance. STAT enables patients to ambulate on a treadmill while wearing a harness connected to an un-weighting system, so their weight can be partially reduced. STAT was shown to improve gait in a variety of neurologic deficits, but has not been used in ALS.

Objectives: To determine safety, tolerability, and efficacy of STAT in ambulatory ALS patients with AD.

Methods: ALS patients (n = 4) who qualified to enrol in this study: had definite ALS; were able to stand and ambulate independently with AD; were able to complete the 25 foot walk test (25FWT) in less than one minute; had a vital capacity (VC) of $\geq$65% predicted. The intervention consisted of 30 minutes of STAT (five minutes exercise and five minutes rest) with 40% of body weight supported. Patients build up their endurance to training as tolerated; walking speed was as tolerated by each patient. Exercise intensity was determined by each patient's rate of perceived exertion (RPE), but not to exceed level 12–13 (mild to moderate) on the modified Borg RPE scale. Six minute walk test on treadmill (T6MWT), ground (G6MWT), 25FWT, ALSFRSR, %VC, Manual muscle test (MMT) of lower extremities, fatigue severity scale (FSS), and RPE during T6MWT were evaluated at baseline (B), 4 and 8 wks post training.

Result: Three patients completed the 8 wks. One patient left after 7 visits due to shoulder pain and difficulty in swinging her leg. T6MWT distance increased 40% ($\pm$7%) and 45% ($\pm$3%), speed increased 31% ($\pm$10%) and 37% ($\pm$14%), RPE decreased 34% ($\pm$10%) and 46% ($\pm$9%), 25FWT time decreased 18% ($\pm$30%) and 26% ($\pm$30%) after 4 and 8 wks respectively. Patient number 3 25FWT time was 5.3 at B, a close to normal value for this test, and did not change. We suspected that G6MWT may be more sensitive than 25FWT for this Pt. G6MWT distances were 315, 427, 445 metres at B, 4 wks, and 8 wks respectively. ALSFRSR scores were 33 ($\pm$6), 37 ($\pm$6), and 36 ($\pm$7);%VC were 87 ($\pm$18), 85 ($\pm$20), and 85 ($\pm$20), MMT were 54 ($\pm$13), 57 ($\pm$14), 57 ($\pm$11), FSS were 37 ($\pm$6), 38 ($\pm$11), and 36 ($\pm$11) at B, 4 wks, and 8 wks respectively.

Discussion and Conclusion: STAT is tolerated with no adverse effects. Active assisted RRPE using STAT shows training effects and may be beneficial for ALS Patients by permitting exercise while protecting from overwork injuries and deconditioning leading to disuse atrophy. STAT needs to be further investigated in a larger clinical trial.

P265 CARDIOPULMONARY RESPONSES TO INCREMENTAL RAMP EXERCISE IN PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: aerobic exercise, exercise testing, oxygen consumption

Background: Scarce data are available about the physiological response to dynamic incremental exercise in patients with motor neuron disease (MND).

Objectives: To assess the cardiopulmonary response to cycle ergometer incremental exercise in a group of MND patients, using ramp protocols routinely performed in the clinical setting for patients' functional evaluation.

Methods: We studied 14 patients affected by MND (12 amyotrophic lateral sclerosis, 1 primary lateral sclerosis and 1 progressive muscle atrophy, age 57 $\pm$ 11 years, 43% males, 10 $\pm$ 9 months from diagnosis) and without associated diseases affecting the cardiorespiratory response to exercise. All patients underwent a ramp incremental cardiopulmonary exercise testing (CPET), with power increments of 5 W/min, 7 W/min, or 10 W/min according to the referred level of patients' habitual activities. Respiratory gas exchange measurements were obtained breath-by-breath using a computerized metabolic cart (Vmax29; Sensormedics; Yorba Linda, CA). Peak VO_2 was the mean VO_2 value observed during the last 30 s of the exercise period. Ventilatory anaerobic threshold was estimated by the V-slope and/or respiratory equivalents methods. Functional Rating Scale (FRS) and resting forced vital capacity (FVC) were also evaluated in the whole study population.

Results: No adverse event was observed during exercise tests. Peak VO_2 averaged 15.4 $\pm$ 5.7 ml/kg/min, corresponding to 61 $\pm$ 18% of predicted maximum, i.e. to a slightly reduced aerobic power; however, the mean VO_2/W relationship slope was 10.2 $\pm$ 1.4 ml/W/min, testifying to a globally preserved

efficiency of the O_2 transport and utilization system. Peak respiratory exchange ratio averaged 1.03 ± 0.05, attesting near-maximal effort attainment. Ventilatory anaerobic threshold was identified in 50% of patients, with VO_2 values averaging 10.7 ± 3 ml/kg/min, i.e. $64 \pm 11\%$ of peak VO_2. Mean peak ventilation was 36 ± 12 l/min, with a VE/VCO_2 relationship slope mean value of 30 ± 6, indicating a preserved ventilatory efficiency. FRS score averaged 37 ± 6, and FVC mean value was 2.6 ± 0.91, equal to $86 \pm 24\%$ of predicted. None of the evaluated parameters was related to time from diagnosis.

Discussion and Conclusions: Patients with MND are usually prevented from aerobic training due to concern about possible negative effects on disease course (muscle fatigue worsening). Our data show that patients with a mean time from diagnosis around 1 year and quite preserved FRS score and FVC can safely perform a near-maximal ramp incremental CPET. The lack of correlation between the evaluated parameters and time from diagnosis suggests an early stage pathophysiology in this study group. In such a clinical setting, the observed slight reduction of peak VO_2 with respect to predicted values in the presence of normal VO_2/W and VE/VCO_2 slope values supports a peripheral, i.e. skeletal muscle, rather than central, i.e. heart and lung, limitation to exercise tolerance. As no data are currently available regarding the effects of aerobic exercise training on exercise tolerance, quality of life, and prognosis of patients with MND, our findings may be used as a rational basis for future studies aimed at addressing these important issues.

P266 EFFECTS OF A HYDROTHERAPY PROGRAM ON FUNCTION AND MUSCLE STRENGTH IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: hydrotherapy, function

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the neuromuscular system resulting in paralysis and ultimately death. Currently, no effective therapy is prescribed for patients; however, several therapeutic strategies are showing promise. Kaspar *et al* indicate that a drug treatment in combination with appropriate exercise may provide the most promising therapy for ALS. While both upper and lower motor neuron dysfunction may contribute to impaired muscle function in ALS, the precise mechanisms of muscle fatigue have not been clarified in this disease. Patients with ALS had less intramuscular phosphocreatine depletion and less fatigue of stimulated tetanic force during exercise compared to controls. Thus, due to the central failure, there was decreased muscle activation resulting in a smaller metabolic demand and less fatigue within the muscle itself. These data demonstrate a major contribution of central factors to muscle fatigue in ALS. The role of physical activity for patients with ALS is controversial. A regular moderate physical exercise program has a short-lived positive effect on disability in ALS patients and should be recommended. Endurance exercise training is known to increase antioxidant capacity in skeletal muscle. In light of a recent report describing increased survival following low-intensity endurance training, it appears that training intensity is an important determinant of survival in the G93A mouse.

People with chronic or long-term physical disabilities, in addition to having basic needs for optimal sensory-motor function and ADL independence, are becoming increasingly interested in physical fitness. The swimming pool program offers a wide range of opportunities, medical and recreational, functional and social, to the consumer with physical dysfunctions. Our objective was to evaluate the effects of a swimming pool exercise program on function and muscle strength of lower limbs in patients with ALS.

This study was performed at the outpatients' Amyotrophic Lateral Sclerosis clinic of UNIFESP/EPM by a physical therapy team from May to December 2005. Twenty-two ALS patients were randomized to receive a moderate exercise program ($n = 11$) or not to perform any physical activity beyond their usual daily requirements ($n = 11$). At baseline and after 6 months, patients were assessed by manual muscle strength testing, and ALS functional rating scale (FRS).

At 6 months, there was no significant difference between groups, although a trend towards less deterioration in the treated group on both scales was observed.

P267 INTRATHECAL BACLOFEN FOR SPASTICITY IN MOTOR NEURON DISEASE: CRITERIA FOR ITB PUMP PLACEMENT

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Keywords: spasticity, intrathecal baclofen, baclofen pump

Background: Management of severe spasticity in Motor Neuron Disease (MND) is often unsatisfactory because patients do not tolerate or are refractory to oral medications. In Primary Lateral Sclerosis (PLS) and upper motor neuron predominant Amyotrophic Lateral Sclerosis (ALS) intrathecal baclofen (ITB) might be an option. Mobility, activities of daily living (ADL), and quality of life can improve, but deterioration is also possible due to unwanted loss of muscle tone. Furthermore ITB therapy is invasive and expensive.

Objectives: To evaluate the usefulness of ITB for intractable spasticity in MND and to define criteria when a permanent ITB pump is indicated.

Methods: Patients were referred by experts of ALS clinics for ITB therapy, hospitalised, and examined by a neurologist, an occupational, a physical, and a speech therapist preoperatively. ALS Functional Rating Scale (ALSFRS-R) and Functional Independence Measure (FIM) scores were taken at baseline, another FIM during ITB therapy. A probatory external baclofen pump, connected with a subcutaneous intrathecal catheter, was placed with local anaesthesia about 40–60 cm above L3/L4 puncture level. ITB was started at a continuous rate of 24 μg/d. The dosage was increased according to clinical signs, oral antispastic medication tapered and stopped. Spasticity was evaluated daily by the modified Ashworth scale; selected ADL, timed 10 m walk, speech and swallowing examined by members of a multiprofessional rehabilitation team. Videographic documentation was made before and under ITB treatment.

Results: From 2007 to 2009 six consecutive patients (4 men, 2 women), mean age 48.5 years, were treated with ITB via probatory external pump. Three were diagnosed with PLS, 3 with upper motor neuron predominant ALS. Mean disease duration preoperatively was 55 months, mean ALSFRS-R at

baseline 29.2. Mean time of probatory ITB external pump was 9.8 days, mean baclofen dosage 44.5 ug. In all patients spasticity was reduced; no side effects were reported. Mean FIM changed from 82.5 to 84.1. One patient did not go on a permanent baclofen pump because swallowing and gait deteriorated under ITB, another patient because ADL and transfers did not improve.

Discussion and Conclusions: The pattern of muscle tone and strength varies substantially and individually in MND patients. Therefore definition of therapeutic targets with the patient and carers is crucial before starting ITB, considering especially swallowing, speech, gait, ADL, head and trunk stability, pain, positioning, transfers. A reliable evaluation requires determination of reproducible items, consistency of investigators and setting, and adequate time period.
ITB is effective but not always beneficial in MND. We recommend analysis of the usefulness of ITB by means of a temporary external pump in an experienced interdisciplinary in-house rehabilitation team. This setting provides an adequate time frame and information level for patients, carers, and professionals to define if placement of a permanent ITB pump is indicated.

P268 TEMPOROMANDIBULAR JOINT DYSFUNCTION IN ALS: A TREATABLE CONDITION

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Keywords: temporomandibular joint, treatment, prevention

Objectives: To describe temporomandibular joint dysfunction (TMJD) in ALS patients and its efficient treatment.

Background: ALS onset is bulbar in approximately 25 to 30% of cases. Except for the oculomotor nuclei, motor impairment is due to the degeneration of motor neurons from 6 cranial nerves at different degrees. To date, except speech therapy, physiotherapy and treatments for excess saliva, few conditions in bulbar patients have been described as being improved by therapy.

Cases: We describe 3 types of patients with TMJD. In the first type, involvement of trigeminal motor neurons is predominant and patients have asymmetrical amyotrophy of the temporalis, leading to a major disequilibrium of the mandibula when feeding, sometimes with dislocation of the joint. In the second type, patients present with significant facial pain due to contractures of the masseters and temporalis, leading to misdiagnosis. In the third type, ALS patients complain of increased dysphagia associated with pain. In those cases a contracture of internal and external pterigoidian muscles have been found. In all three types, the patients were female and the consequence of the TMJD was weight loss, one important prognostic factor in ALS. For all the patients, the complaints completely disappeared with specific physiotherapy. In some cases, after several months, the same TMJD recurred and was successfully treated again.

Discussion: TMJD is treatable condition in bulbar ALS patients that may present in different ways but is always responsible for impaired quality of life and weight loss. For those reasons TMJD should be recognized and treated as soon as possible. While some presentations could be misleading, such as intense facial pain, physicians should be aware that facial pain should not *a priori* lead them to exclude ALS.

P269 NON-SURGICAL TREATMENTS FOR DROOLING IN MND/ALS: A SYSTEMATIC REVIEW

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Keywords: saliva, systematic review

Background: Bulbar weakness is the main presenting feature in approximately 25% of patients with MND/ALS. Drooling of saliva is a distressing symptom associated with bulbar dysfunction. This can have important physical, psychological and social effects on the individual and their family. Patients can experience thick mucoid secretions pooling in the oropharynx and/or thin watery saliva which leads to drooling. It is important to identify the type of difficulty experienced to guide intervention. Treatment approaches vary, therefore to improve patient care, disease-specific evidence-based guidelines are needed.

Objectives: To assess the evidence to support current non-surgical treatments to reduce drooling in MND/ALS.

Methods: A systematic review of the treatment of drooling was carried out. Inclusion criteria were: 1) evaluations of non-surgical interventions to reduce thin saliva in MND/ALS; 2) studies of more than two subjects; and 3) published in English language. The search strategy was divided into four steps 1) electronic search of four clinically relevant databases; 2) hand searches of all 19 International ALS/MND symposium journals; 3) an email request for unpublished studies sent to the 16 MND care centres in the UK and Ireland; and 4) hand searching of reference lists.

Results: Of the 64 published reports identified on the basis of title and abstract, only 15 met the inclusion criteria. The methodological quality of these studies varied, only one was a randomised controlled trial. Of the 15 studies, 4 reported the use of radiation, 8 studies the use of botulinum toxin injections, 1 reported a combination of radiation and botulinum, 1 a comparison of botulinum and amitriptyline and 1 the sublingual administration of atropine. Sample sizes were small ($n=4$ to $n=20$). Outcomes included the weight of cotton wool, number of tissues used and patient rating scales. The average length of follow up varied between 2 and 80 weeks. Although all studies reported the intervention tested to be effective only 5 studies showed statistically significant changes.

Discussion and Conclusions: Although many authors in the studies reviewed claimed that botulinum toxin injections and radiation are effective in reducing saliva in patients with MND/ALS, it has not been possible hitherto to demonstrate this with a definitive randomised controlled trial. While lack of consensus on the best treatment available for salivation in MND suggests that a trial could be justified on the basis of equipoise, recruiting sufficient patient numbers to detect important differences might be problematic. We argue that to achieve this goal, a consensus needs to be built on how to standardise treatments, and identify the most patient-centred and robust outcome. This will then allow for such treatments

to be tested in an ethical manner in multiple centres to allow meaningful statistical interpretation.

P270 TREATMENT WITH CLONIDINE THROUGH INHALATION TO REDUCE THE SIALORRHEA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: sialorrhea, clonidine, aerosol

Background: Fifty percent of patients with amyotrophic lateral sclerosis (ALS) experience problems handling serous saliva and 20% fail to achieve adequate control of sialorrhea with anticholinergic medications, or experience intolerable adverse effects from these drugs.

Objectives: This Open-label prospective study of clonidine administered through inhalation for the treatment of sialorrhea in patients with ALS was designed to determine safety as well as efficacy.

Methods: ALS patients with sialorrhea refractory to medical therapy were enrolled in this study to be treated once a day with a clonidine aerosol (0.07%). Primary outcome was rate of responders (improvement >50% on visual analogue scales (VAS) of severity and disability of sialorrhea) after 1 month of treatment. Other outcomes included subjective (drooling and quality of life questionnaires) and objective (number of paper handkerchiefs used) evaluations. Safety evaluations included daily blood pressure measurement.

Results: Fourteen ALS patients were included. At 1 month the rate of responders was 80% with a mean reduction of 70% in severity and disabling VASs. Thirteen patients (92%) reported some benefit with drooling reduction. In objective measurements there was a reduction over 60% in the number of handkerchiefs used. Onset of effect occurred few minutes after the inhalation and the duration of the effect was about 24 h. Most patients reported better quality of living. The most frequent side-effects were viscous saliva. There were no changes in blood pressure or cardiac rate. At 3 months, there was still a positive effect in all outcomes. All patients except one manifested their willingness to continue the treatment.

Discussion and Conclusions: Clonidine aerosol seems to be effective and safe to treat sialorrhea in ALS. It is suggested that the salivary reduction induced by clonidine may be mediated by a stimulation of alpha 2-adrenoceptors, which exerts an inhibitory control of the parasympathetic activity on the salivary glands.

P271 DIAGNOSIS AND TREATMENT OF DYSPHAGIA IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: dysphagia, videoendoscopic evaluation of swallowing (VEES)

Background and Objectives: ALS is the most common neurodegenerative disease of the motor system. Dysphagia is a frequent symptom of ALS and results in reduction of life expectancy/quality. At the beginning of the disease 20–30% of the patients demonstrate dysphagia whereas in later stages nearly 100% are affected. We started to use standardized testing for dysphagia in ALS to determine if compensatory/restitutional methods, dietary modifications, augmented feeding techniques, pharmacological interventions or surgical options are necessary (1).

Methods: The assessment comprises a clinical examination and VEES which is useful for educating patients and carers, as they clearly reveal the process of swallowing and demonstrate the risks involved in swallowing. It enables direct observation of pre-deglutitive and post-deglutitive actions within the pharynx and larynx. On the basis of a clinical examination and VEES observations, a treatment plan for swallowing therapy can be established or a decision can be made concerning further alimentation.

Results: Dysphagia in ALS can either result within a pseudobulbar paralysis or from muscular weakness innervated by trigeminal (V), facial (VII), hypoglossal (XII), glossopharyngeal (IX) or vagal (X) nerves. Swallowing is generally conceptualized as occurring in several distinct phases. In the following we describe the typical disorders during these phases: 1) In the pre-oral phase muscular weakness leads to failure of bolus breakdown/manipulation, preparation of solids and decreased bolus control resulting in premature spillage, pre-swallow pooling and post-swallow residuals in the oral cavity and/or the pharynx; 2) During the oral phase weakness of the tongue leads to increased bolus transition time, incomplete oral bolus transport and to oral/sublingual post-swallow residuals, whereas a delayed swallow reflex results in premature spillage, penetration and aspiration; 3) In the pharyngeal phase decreased base of tongue to the posterior pharyngeal wall approximation as well as incomplete larynx elevation results in post-swallow residuals; reduced pharyngeal motility and supraglottic compression lead to intra-swallow penetration/aspiration and post-swallow residuals; impairment in opening the upper oesophageal sphincter results in post-swallow residuals and aspiration; vagal deficits results in decreased effectiveness of cough on aspiration (motor) or silent aspiration (sensory); 4) The esophageal phase is not affected in ALS.

Discussion and Conclusion: Optimal care of patients with ALS requires attention to laryngeal and pharyngeal symptoms. With the assistance of a speech and language therapist

the patient can overcome mild to moderate degrees of dysphagia. The adoption of special techniques can aid swallowing. In advanced stages augmented feeding strategies should be considered (1).

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P272 QUANTITATIVE VOICE ANALYSIS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH BULBAR ONSET

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Background: Patients with early symptoms of bulbar ALS are usually referred to the otolaryngologist without a diagnosis. One of the earliest symptoms of bulbar involvement in ALS is voice deterioration. Voice assessment in ALS patients has been done frequently mainly by perceptual analysis. The objective parameters, including acoustic measures and videostroboscopy analysis, have been measured only in a few, small series of patients.

Objectives: To determine the vocal parameter characteristics for bulbar ALS patients.

Material and Methods: ALS patients were diagnosed according to El Escorial criteria (1998) in the Department of Neurology, Jagiellonian University Medical College in Krakow, between 2007–2008. The voices of 23 bulbar ALS were studied in the Department of Otolaryngology using a quantitative voice analysis system (with acoustic parameters) and laryngovideostroboscopy examination (LVSS). Measurements were compared with those obtained in 20 normal subjects (controls).

Results: Ninety-six per cent of ALS patients studied complained of dysarthria, 67% of dysphagia, and 56% of dysphonia. Hoarseness (37%), hypernasality (32%), asthenicity voice (31%) were the most common voice perception scores, in addition to being dysarthric. In 69% of all ALS patients timing of phonation was characterized by frequent complete voicing interruptions during running speech and vowel prolongation. The most common findings on LVSS were slowness of one/both vocal fold (VF)-87%, incomplete closure of VF-63%, bowing VF-30%, decreased VF abduction-27%, hyperadduction of VF-23% as well as a mucous pooling-19%. The shorter phonation time (PT) was detected (98%) as well as abnormal frequency of the voice (range: 60–140 Hz) (86%). The analysis of the amplitude and frequency perturbation of sound (Jitter and Shimmer parameters) have presented statistical significance difference ($p < 0.01$) as compared to the control group. Phonatory instability was also presented.

Discussion and Conclusion: We have previously stressed that hyper-/hyponasality, articulation defects, voice harshness and breathiness as well as swallowing difficulties should all be viewed as possible early signs of ALS and may allow the otolaryngologist to be the primary diagnostician. This study shows that on LVSS, slowness of one/both vocal folds, was the most frequent observation in bulbar ALS cases. These patients' voices were characterized with abnormal Jitter and Shimmer and very short phonation time.
We suggest that in bulbar ALS patients careful examination of the speech quality, a videostroboscopy examination as well as an acoustic analysis should be performed to measure voice disturbances due to disease.

P273 NEW URINARY MANAGEMENT SYSTEM FOR MALE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: urinary management, QOL, burden

Background: A distinctive characteristic of Amyotrophic Lateral Sclerosis (ALS) is that it does not affect urinary function, but systemic muscle weakness impairs the movements necessary for maintaining normal urinary habits. In a survey of the actual situation of ALS patients and the level of burden on the caregiver, the top 3 burdens identified by caregivers were suction of mucous and saliva (44%), defecation (36%), and urination (33%). In particular, a heavy burden associated with multiple episodes of night time urination was identified. Urination is a very private matter, and in providing care or assistance, it is important that we allow the patient to retain his dignity, especially in patients with ALS, because their intellectual function and personality are preserved.

Objectives: We report our experience of using a new system for urinary management in male ALS patients with urinary care problems.

Methods: A new urinary management system for males (afex®) was originally developed to manage incontinence associated with complications of prostate cancer surgery. The system comprises several components which can be combined in different ways to match the needs of ambulant users, wheelchair users, bedridden users, and users of a handheld urine receptacle. The key component of the system is the "receptacle", consisting of an outer layer of high-density polyethylene and an inner layer of soft PVC which has multiple perforations allowing urine flow. It is designed to prevent backflow to some degree. The receptacle has a diameter of 45 mm and does not put pressure on the penis.

Results: In the present study, we tested the system as adapted for wheelchair and bedridden users, and in both situations it resolved the problems the patients had faced prior to using the system, and improved their QOL. Patients have also used the device successfully when going out of the house and no longer need to worry about how to handle urination when away from home. There were no adverse events preventing continued use of the system and the patients were using them comfortably. Sometimes while at work, the patient was bothered by an ammoniac smell, but was able to solve this problem by placing a ceramic, non-woven sheet with deodorant capability over

the receptacle. There was much less skin trouble than with a condom catheter and it was much more comfortable to wear. Use of this system incurs an additional annual cost to the patient, but both patients were very satisfied and intend to continue using the system. For the patient who had used diapers, a cost saving was also achieved.

Conclusion: The new system for managing male urinary continence is very useful for ALS patients at all stages of the disease, and in the diverse situations of their daily lives.

P274 MULTIDISCIPLINARY CARE FOR MOTOR NEURONE DISEASE

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Keywords: multidisciplinary care, quality of life, rehabilitation

Background: Multidisciplinary care (MDC) is an important means of symptomatic and supportive management for motor neurone disease (MND) but the evidence base for its effectiveness is unclear.

Objectives: To assess the effectiveness of MDC in adults with MND, especially in the types of approaches that are effective (settings, intensity) and the outcomes that are affected.

Methods: *Search strategy:* We searched the Cochrane Neuromuscular Disease Group Trials Register, Cochrane Central Register of Controlled Trials (CENTRAL 1966-present), MEDLINE (1966-present), EMBASE (1980-present), CINAHL (1982-present), AMED (1985-present) and LILACS (1982-present). *Selection Criteria:* Randomised and controlled clinical trials (RCTs, CCTs) that compared MDC in MND/ALS with either routinely available local services or lower levels of intervention; or studies that compared MDC in different settings or at different levels of intensity. Trials of 'other designs' (ODs) (such as observational studies) were also included with the understanding that such trials could only be of limited contribution to the best evidence synthesis. *Data collection and analysis:* A "best evidence" synthesis based on methodological quality was performed. Studies were grouped in terms of setting and intensity (high or low) of therapy.

Results: No RCTs /CCTs were identified. Five ODs, three of 'low quality' and two of 'very low quality' based on methodological quality assessment were analysed. There was 'very low level quality' evidence for MDC in low intensity outpatient settings for improvement in the mental health domain of quality of life (QoL). There was 'low level quality' evidence for hospitalisation in terms of reduced hospital length of stay (LOS) and fewer re-admissions; and conflicting evidence regarding survival outcomes (two studies demonstrated improved survival and one did not). As for high intensity MDC, there was 'very low level' evidence for improvement in impairment and disability.

Discussion and Conclusions: In the absence of RCTs/CCTs, the "best" evidence to date is based on 'low' to 'very low quality' ODs. These suggest an advantage for mental health domains (only) of QoL, reduced hospitalisation, and improved disability for people with MND. The evidence for survival is conflicting. These conclusions are tentative, and need further research into appropriate study designs; outcome measurement; carer needs; and the evaluation of optimal settings, type, intensity/frequency and cost effectiveness of MDC in this population. Future research should focus on observational designs (Practice Clinical Trials), to assess care and outcomes in "real-life" settings. The interface between neurology, rehabilitation and palliative care should be explored to provide long-term support for MND.

P275 INDEPENDENT LIVING FOR SPINAL MUSCULAR ATROPHY PATIENTS: MENTAL CARE FOR PATIENTS LEAVING LONG-TERM HOSPITALIZATION

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Keywords: SMA, long-term hospitalization, independent living

Background: In Japan, patients with degenerative motor neuron diseases such as Amyotrophic Lateral Sclerosis (ALS) or Spinal Muscle Atrophy (SMA) have tended to be regarded as people with a critical illness. As a result, such patients have sometimes had to live in facilities or hospitals for years or even decades if their families could not take care of them. Moreover, some patients have had to live in the hospital from childhood in order to attend school there. In a recent change, however, such patients are beginning to be considered as people with a severe disability, and they are being given the opportunity to live independently outside of hospitals or institutions. Nevertheless, no manual or method has yet been established for providing the necessary mental support to such patients, both before and after leaving the hospital, to enable them to live independent lives.

Objectives: We attempt to outline the mental support necessary for enabling long-term hospitalized patients to leave hospitals and live independently. These patients should receive counselling and training to learn how to communicate in society, go shopping, do the washing, cook their meals and perform other aspects of independent living.

Method: To investigate the life of SMA patients after long-term hospitalization, we conducted interviews with 5 SMA patients living independently in society. We also conducted a questionnaire survey of disabled people who live independently in society after leaving institutions or hospitals. In addition, to make a model case, we conducted research about a long-term hospitalized patient's actual departure from hospital.

Discussion and Conclusions: It seems that, before leaving the hospital, patients worry about their future caretakers and economic condition, and, after leaving the hospital, they worry about their relationships with their caretakers. When patients with a neurological disorder are in a bad condition, they absolutely require proper care from caretakers. In such situations, they seem to worry whether caretakers can understand how to take care of their special neurological disorder, because few caretakers have experience with severely disabled patients. Taking care of patients with a neurological disorder is extremely confusing, because it is difficult to understand their social life and how to talk with them in some situations. However, they can be independent with the support of others. Many patients want to live in their own home, not in a hospital or an institution. Mental support is necessary so that all patients who want to live independently in society can do so.

P276 'WHEN SOMEONE CLOSE HAS MND': NEW RESOURCE FOR CHILDREN

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Keywords: interactive, workbook, children

Background: MND is a distressing disease for children to grasp. They may feel inquisitive, but fearful of asking questions that might upset anyone. When reviewing MND Association literature for children, we wanted to make sure new literature reflected the needs of the child.

Objectives: To create a resource to support children from 4–10 years affected by MND in their family. Acknowledging that 'one size doesn't fit all', we wanted a resource that could be adapted and personalised to meet individual needs, and would create opportunities for children to ask questions and take part in discussion.

Methods: A collaborative project between the MND Association and Trinity Hospice, London. Extensive evidence based research (1–3) established how children learn, develop thinking skills, reflect upon information presented to them, and mechanisms children use to help them cope.

Results: We developed the interactive work book 'When someone close has MND' as a way of encouraging children to identify and develop their own coping strategies. The introductory section of the work book includes information for adults about how they can best support a child when an adult is seriously ill (4–6). As it wasn't appropriate to involve children between 4–10 years to participate in the development of this resource, we approached people aged 15–23 years who had previously lost someone close to MND.
This brightly illustrated resource is presented in loose leaf format so adults can introduce information as and when it becomes timely, relevant and appropriate, allowing pacing of information, which is best practice when supporting a child when someone close to them is seriously ill. This format allows it to be personalised for individual needs.
This new resource will enable people affected by MND to talk about the things that really matter, one step at a time, to meet the needs of the child. We will measure the success of this resource by seeking feedback from adults and children who request it.

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P277 THE EXPERIENCES OF HOSPICE NURSES WHO CARE FOR PEOPLE WITH MOTOR NEURONE DISEASE

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Keywords: caring, nursing, counselling

Background: The study has been designed to examine the relationship which develops between the person with MND and their nurses. The patients reported that the relationship they had with their nurses sustained them and fostered a sense of hope despite the ongoing nature of their difficulties. This study examines the experiences of Registered General Nurses (RGN) who work in a hospice and have cared for people who have Motor Neurone Disease (MND).

Method: A qualitative approach was used to capture the experiences of a purposeful sample of RGNs who had the required experience. Six nurses were interviewed, using a semi structured interview. The interviews were recorded, and then transcribed. The data was analysed using Interpretive Phenomenological Analysis (IPA).

Results: Six themes emerged from the data; they were: 1) the nature of the disease progression for the patient with MND; 2) the nature of suffering in the patient with MND; 3) the impact that communication impairment had on the nurse-patient relationship; 4) the nursing approach used and the skills required to care for the patient with MND; 5) conflicts in care which arose for the nurses when caring for a patient in a hospice environment; 6) the challenges and the rewards which arose for the nurse when caring for the patient with MND. On deeper analysis a master theme emerged linking all the other themes. This was the nurse-patient relationship.

Discussion and Conclusion: Findings demonstrate that the nurses in the study found the experience of caring for the patient with MND challenging and very satisfying. The intensity and complexity of the experience, combined with the difficulties of working with people who have severe communication impairment meant that they had to call on all of their nursing skills to meet the challenges. The nurses found that working in supportive nursing and multidisciplinary teams encouraged them to use skills of emotional intelligence and intuition. This experience has meant that they are able to establish deeper, more satisfying relationships with other patients.
Recommendations from the study include the recognition that nurses are able to establish and maintain therapeutic relationships with their patients. This leads to positive outcomes in patient care, including support during times of distress and emotional anguish. Nurses would benefit from using a model such as person-centred counselling to understand the importance of the relationship, in supporting adaptation to illness, disability and dying. Nurse educators need to focus on fostering broader nursing expertise than competencies and skills with an acknowledgement that depth of relationship improves patient outcomes.

P278 CARING FOR THE CAREGIVER PART 2: EVALUATION OF AN ALS CAREGIVER ASSESSMENT FORM FOR A MULTIDISCIPLINARY ALS CLINIC

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Keywords: caregiving, assessment, quality of life

Background: The family caregiver is heavily involved in direct and indirect caregiving activities of the patient with ALS. These activities include assisting with activities of daily living, involvement in medical care planning and decision making, use of medical devices, and providing emotional support. Caregivers are often burdened by the volume and intensity of the ALS experience and require professional assessment and intervention.

Objectives: We will report on the evaluation of a Caregiver Assessment Form in a multidisciplinary ALS Clinic.

Methods: A team of ALS clinicians and researchers used the evidence based practice (EBP) method to develop a Caregiver Assessment Form containing questions about demographics, concerns with caregiving tasks, health and well-being, and supportive activities. The form was mailed to caregivers prior to the ALS Clinic visit, and the completed questionnaire was made available to clinicians during clinic. Caregiver exit interviews were conducted to evaluate the content of the instrument and its perceived ability to meet caregiver needs. Interviews with clinicians were completed to evaluate the clinical utility of the instrument for ALS Clinic.

Results: The assessment form was completed by 39 (90%) of the caregivers who were asked to do so. 84% of caregivers found the tool easy to use. 80% believed that content in the form was appropriate. Completing the assessment form helped 46% identify caregiving concerns about which they were unaware. 92% felt that their concerns were addressed satisfactorily. 92% thought that the addition of a caregiver assessment form would improve the support received from the clinical team. The evaluation of the form by the clinical team members showed that 79% felt that the form helped them to assess the needs of the caregiver. Clinicians indicated that they would use the tool to get the "big picture" of what the caregivers are dealing with, and to help focus the visit on high concern areas related to patient management. A time constraint was identified, with only 50% of clinicians reporting that they were able to review the tool prior to visiting the patient and caregiver.

Discussion and Conclusions: The caregiver assessment form was positively received by caregivers and the clinical team. The addition of a caregiver assessment form to a multidisciplinary ALS clinic appears to have enhanced the caregivers' and the clinicians' ability to identify caregiver needs as they relate to the care of the patient with ALS. A caregiver assessment form is an intervention that can be used to support caregivers of patients with ALS.

P279 THE PERSONAL EXPERIENCE OF CARERS OF INDIVIDUALS WITH MOTOR NEURONE DISEASE AND THEIR EXPERIENCES OF SERVICES

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Keywords: caregiving, couples, interpretive phenomenology

Background: Carers play an essential role in the lives of people suffering from chronic health problems. Understanding the concepts related to caregiving experiences and the relationships among them can enable us to better address the needs of caregivers. MND raises many of the issues relevant to caring for people with chronic disabling conditions, and yet there is a lack of research exploring the experiences of those assisting someone with MND. The findings to date are diverse and sometimes contradictory.

Objectives: To find out what it is like to experience having a partner with MND, how services are experienced, and the meanings people give to these experiences. To understand, interpret and contextualise the detailed accounts of eight individuals. To use the knowledge gained from this study to improve the provision of services provided to individuals with MND and their carers, and enhance the general body of research on carers and their needs.

Methods: Eight semi-structured interviews were carried out and transcripts were analysed from an Interpretative Phenomenological perspective.

Results: The main themes are: 1) impact on life, which is broken down into: needing to be strong for partner, concern for partner's safety, impact on relationship, anger and frustration, physical tiredness, and uncertainty around the future; 2) adjusting to the situation, which includes: experience of services, a problem-solving approach to practical difficulties, living day-to-day, adapting to lifestyle changes, and ability to remain positive.

Discussion and Conclusions: From the results, the need to be strong for partners was evident, as well as worries for partners' safety. Physical tiredness as a result of caring for their partners and uncertainty around the future were also themes in all accounts. Individual differences were evident around the impact on their relationships; some experienced a loss of intimacy more than others and the role of caring for their partner appeared to change the dynamics of the relationship for some and not others. Anger and frustration were also clear in some of the descriptions, but not all. Experiences of services were varied, although most people described negative experiences around receiving the diagnosis and felt well supported by the professionals at the specialist MND clinic. A problem-solving approach to practical difficulties appeared to help in coping with the emotional impact around their partner's deterioration, as well as living from day-to-day. Participants varied in their ability to adapt to lifestyle changes, and in their ability to remain positive.
This study has highlighted some important aspects of the participants' perspectives in relation to having a partner with

MND, and it is clear that services around people with MND need to review the support offered to carers.

P280 WHY DON'T PEOPLE WITH ALS/MND ACCESS SOCIAL SERVICES CARE AND WHAT IMPACT DOES THIS HAVE ON CARERS?

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Keywords: social services care, carer burden, support

Background: A descriptive audit of the key characteristics of the MND population in a large UK specialist MND clinic was undertaken in 2008 as part of a larger study exploring the personal experience of living with MND (1). The audit revealed that a significant number of patients and carers were not accessing external caring support provided by local social services.

Objectives: The aims of this part of the study were to identify the reasons for limited care input from social services and to explore carers' experiences of care services.

Methods: Narrative interview data were collected from people with MND (n = 24) and their spouses or partners (n = 18). Thematic analysis has been aided by NVIVO.

Results: A number of themes relating to experiences of caring have been highlighted in the interview data and a variety of reasons for limited external care provision have been reported. These include carers' and patients' lack of information or understanding of the roles of services; issues around the allocation of social workers; carers' and patients' desire to maintain a sense of normality; reluctance to surrender privacy or control of home lives. Carers report feelings of anxiety or guilt when considering accessing care outside of the home. A number of individuals have reported lack of consistency and organization of care agencies, poor standards of care and limited understanding and training in MND. Carers report experiencing significant levels of emotional and physical exhaustion and a lack of social and psychological support, training and information available to them.

Conclusions: This study has provided detailed information regarding the uptake of care services by the current MND population followed up by a specialist UK MND clinic. It explains to some extent why patients and carers are not accessing services and highlights a number of the issues faced by carers of people with MND.

Reference:

1. O'Brien M, Whitehead B, Mitchell D *et al*. ALS 2008; 9 (Supp 1): 147

P281 SELF-PERCEIVED BURDEN AND A SPIRITUALITY OF INTERDEPENDENCE

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Keywords: burden, spirituality, interdependence

Background: People living with ALS/MND can sometimes perceive themselves as burdensome to their families and society. Their sense of self is diminished by the many losses that come with the diagnosis and disease. This can lead to spiritual distress. They may feel unworthy of the care of others or do not want to trouble others with their problems. They may also be afraid of suffering. A spirituality of interdependence can offer a new direction by helping people with ALS/MND to understand their experience in the context of relationships rather than individualism.

Objectives: This presentation will explore the phenomenon of feeling like a burden from a spiritual perspective. Reasons for feeling burdensome to others will be explored and a framework of spiritual care interventions based on a theology of interdependence will be offered.

Methods: A review of the psychological literature on self-perceived burden will be presented. Case studies highlighting the experience of feeling burdensome to others will be shared. Some of the possible outcomes for the person with ALS/MND will be identified. Feeling like a burden can lead to varying degrees of spiritual distress including the desire for altruistic suicide.

Results: Spiritual care interventions can address the feeling of being a burden to others. Spiritual discussions can include exploring a person's sense of self - Who am I now with this ALS diagnosis? - as well as a person's sense of relationship with others. A theology of interdependence will explore what it means to "bear one another's burdens" (Galatians 6:2). This passage from Scripture means not merely to "tolerate" or "put up with" but rather, to "uphold lovingly." To uphold lovingly one who is struggling with feeling a burden can be done by significant others as well as a community. Cultivating community requires that people with ALS and their families are open to community and build their relationships.

Discussion and Conclusion: It is difficult to come to a place of acceptance of one's own vulnerability and interdependence with others, especially in the North American culture which values individual autonomy so highly. Being in relationships with others emphasizes a theology of interdependence: we give and receive in our human interactions. The blessing of community whether it is a health care, ALS or faith community will be discussed. Other theological themes will be presented including some insights from Jewish theologian Emmanuel Levinas and philosopher Paul Ricoeur.
Feeling a burden is a reality for some people living with ALS/MND. Spiritual resources can be drawn upon to help people with ALS/MND address this struggle, support their sense of hope and improve their quality of life.

P282 SATISFACTION OF LIFE AMONG PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS, WITH AND WITHOUT LONG-TERM MECHANICAL VENTILATION: A PILOT STUDY

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Keywords: depression, life satisfaction

Background: Previous data suggest that life satisfaction may be poor for individuals with ALS who are administered tracheostomy and long-term mechanical ventilation (LTMV) at the time of a respiratory crisis. In such a situation, this aggressive ventilatory approach may lead to an uncertain and sometimes unwanted prolongation of life. Moreover, the dependence on ventilatory support may determine a dramatic physical, psychological and economic burden on family and caregivers causing a sense of guilt in patients' psychological status.

Objectives: To further investigate the impact of tracheostomy on patients' mood tone, satisfaction of life, and religious beliefs on this condition.

Methods: We administered the Beck Depression Inventory (BDI), Life-Satisfaction Questionnaire (LSI-11) and Royal Free Interview (RFI) to 13 ALS patients with LTMV compared to 32 ALS patients without LTMV, of comparable for demographic variables. All patients (45) were non-demented.

Results: Despite major physical limitations, no significant differences ($p < 0.05$) were found between ALS patients with LTMV and ALS patients without LTMV in terms of BDI, LSI-11 and RFI scores. BDI data show only 15% of our LTMV patients and 9% of patients who did not receive LTMV admitted severe depressive symptoms. Satisfaction of life appeared highly comparable with the normal population for both ALS groups. The two ALS groups were similar for religious beliefs.

Discussion: Although depression can occur in ALS patients, good satisfaction with life is observed both in ALS groups, with and without LTMV. Religious beliefs and probably social support are positive factors for both groups of patients. Our findings might help physicians to face the challenging ethical dilemma associated with the management of ALS patients who acutely require LTMV or who require detailed information about LTMV treatment. Questions can arise about the psychological impact of unplanned tracheostomy.

P283 THE ROLE OF BURDEN, DEPRESSION, ANXIETY AND SOCIAL SUPPORT IN ALS CARE

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Keywords: care burden, caregivers, social support

Background: The natural history of ALS patients is associated with an increasing burden experienced by their caregivers. There are still few studies that have investigated the emotional impact of ALS care.

Objectives: We aim to evaluate the relationships between burden and psychological aspects in ALS caregivers and the impact of social support.

Methods: We conducted a cross-sectional study among 30 ALS caregivers, using Zarit Burden Inventory to assess burden of care, Beck Depression Inventory-II for depression, State-Trait Anxiety Inventory for anxiety, Social Support subscale from the McGill Quality of Life Questionnaire and ALSFRS-r for patients' severity of disease.

Results: Caregivers' burden, depression and anxiety levels were positively related to each other, with a negative correlation with perception of social support. Patients' illness severity was correlated with caregivers' burden, anxiety and somatic expression of depression. Caregivers expressed worries only for their own health conditions.

Discussion and Conclusion: In our study we showed that burden is directly related with psychological difficulties in ALS caregivers, increasing together with the patient's disability. Furthermore, social support provides a positive effect to limit the worsening of burden, depression and anxiety experienced by the caregiver.

P284 EYE-TRACKING COMMUNICATION SYSTEM: IMPACT ON QUALITY OF LIFE AND MOOD IN PATIENTS WITH ALS IN LOCKED-IN SYNDROME

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Keywords: eye-tracking system, communication, quality of life

Background: During the course of ALS the ability to communicate is progressively impaired, up to the complete loss of useful communication. There are no studies on quality of life (QoL) and psychological aspects related to the eye-gaze system of communication in ALS patients in locked-in syndrome.

Objectives: To assess the impact on QoL and mood of an eye-tracking system (ETS) for communication in a series of ALS patients with locked-in syndrome.

Methods: Nine tracheostomized ALS patients (7 males; mean age 43.8, range 29–61), without clinical cognitive impairment, who use an ETS daily, underwent a questionnaire specifically created by our ALS Centre, focused on the qualitative-organizing aspects from the patients' perspective; they were also administered the McGill Quality of life questionnaire (MQoL), the Zung Depression Scale (ZDS), and the Satisfaction with Life Scale (SWLS). The questionnaires were sent to the patients and returned by email. MQoL, ZDS and SWLS had already been administered to the patients 2 to 5 months before tracheostomy.

Results: All patients were able to fill in the questionnaires using the ETS without the help of their caregivers. All patients stated that the use of an ETS improved their life conditions and also their caregiver's activity; moreover patients felt that after the availability of the ETS they could be in contact with the surrounding world again. The major problems reported by patients were tiredness related to the prolonged use of the

ETS (7 patients) and early difficulties in using the ETS related to their poor previous experience with computer technologies (4 patients). In 7 patients QoL, as well as level of depression, improved after the use of the eye-tracking system; only two patients were still depressed and reported a low level of QoL, although after the use of ETS both ratings improved compared to the evaluation performed before tracheostomy. SWLS score showed a high variability.

Conclusion: Eye-tracking systems appear to be effective in improving QoL and mood in locked-in ALS patients and their caregivers. Moreover, they effectively increase the communication between patients and caregivers and give to patients the feeling of being able to better control their lives.

P285 A COMPARISON OF EYE-GAZE COMMUNICATION SYSTEMS AND A PROTOCOL FOR ASSESSMENT

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Keywords: eye-gaze, communication, technology

Background: Speech pathologists who work with patients diagnosed with ALS/MND frequently recommend Augmentative/Alternative Communication equipment. These recommendations should identify the most appropriate system based on trials with several different speech generating devices. With regard to eye-gaze technology, new systems have recently been introduced and few professionals have had hands-on experience with all of them. Each of these systems employ complex eye-tracking technology which can be impacted by a number of variables. In addition, eye-gaze system set-up and troubleshooting can be more involved than that with other systems or access methods. When eye-gaze is determined to be the most reliable access method for a patient who requires a speech generating device, one must take many different factors into consideration to ensure user success.

Objectives: To describe the differences between different types of video-based eye-tracking technology, introduce a protocol for eye-gaze evaluations and discuss patient/caregiver feedback.

Methods: To clearly define the different eye-gaze technologies available, a review of literature on eye tracking methodology will be completed. A protocol for conducting eye-gaze evaluations with ALS/MND patients will be utilized to assess patient interaction with 6 different eye-gaze communication systems. Each system will be rated according to a number of variables including the following: 1) simplicity of calibration, 2) speed and accuracy of communication at word, phrase and sentence level (communication software features & functionality), 3) functionality and ease of use for internet access, email and other applications, 4) length of time needed from initial introduction to functional use of the system.

Results: How differences in eye-tracking methodology affect the patient evaluation process and patient satisfaction will be discussed. Patient feedback and anecdotal evidence will be reported. Expected outcomes include varied patient success and preference based on ease of use, comfort/experience with technology, educational and financial status, expectations and communication, environmental control and computer access needs. Results of a patient/caregiver questionnaire will be presented to summarize concerns specific to each individual.

Discussion and Conclusions: Based on our experience, eye-gaze systems can be an ideal communication solution for patients with ALS provided a thorough assessment is completed and the most appropriate system is obtained. It is recommended that speech pathologists working with this patient population become familiar with the different types of eye-gaze technology and perform evaluative trials whenever possible.

P286 COMMUNICATION: HOW DO PEOPLE REALLY FEEL ABOUT THE LOSS?

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Keywords: communication, feelings, therapy

Background: The author's experience with MND and other neurological progressive disorders (PND) led to the conclusion that what is shown to others by those who are communication impaired is only the tip of a deep pain and despair which the client is unable to fully articulate. Communication is not just a means of satisfying wants and physical needs, but is also necessary to have some control over our lives and what happens to us.

Objectives: This study aimed to research the lived experience of communication impairment and to attempt to uncover the true feelings of clients and carers about the communication problems in MND and other PNDs. A deeper understanding of the problems should lead to more effective Speech & Language Therapy management and treatment services, which would be based on the expressed needs and views of the people using them.

Methods: A qualitative approach which used in-depth interviews with 4 people with MND and 2 others who had progressive neurological conditions, and their main informal carer. These interviews were taped and transcribed and content analysis used to identify themes which were further allocated to overt and covert themes. Because many of the clients had extremely limited communication, 'vignettes' were written from the interview with each participant to attempt to describe their feelings. This was verified as correct by one patient and carer as a control to ensure the extracted meanings were correct.

Results: Both overt and covert themes were uncovered. Overt themes involve those which became apparent from the conversations with clients and carers. Covert themes are those which were not actually discussed openly, but were meanings embedded in discussion, and uncovered by continuous analysis of the transcripts.

Discussion and Conclusions: Overt themes included the overall difficulties of loss of communication and the clients' consequent feelings of guilt and self-blame; covert themes related to the reactions to this loss and the adjustment and coping strategies utilised by both client and carer. Functional and emotional communication were major issues in this study, but also an important quality of life issue was uncovered which relates to social communication, and how the loss of this leads the person to a depleted quality of life and on a pathway to isolation. This led to curtailment and eventual inability of the person not only to control their lives and express their emotions but also to play a part in society. This study has given a greater understanding of the true feelings of

both client and carer with regards to communication and has also altered the therapeutic approach offered, by the introduction of a simple 'life map' method of managing communication deterioration.

P287 SPEECH INTELLIGIBILITY AND MARITAL COMMUNICATION IN MND

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Keywords: speech intelligibility, marital communication, augmentative and alternative communication

Background: The onset of a progressive, terminal illness such as Motor Neuron Disease (MND) inevitably results in physical and communication disabilities that impinge on the individual's ability to remain functionally independent. The loss of speech as a result of dysarthria, a motor speech disorder, is one of the most profound changes that the person with MND will experience. The decline in the individual's speech intelligibility, which negatively influences communication effectiveness, implies that in 80% of cases alternative and augmentative communication (AAC) strategies are required to support the daily communication needs of individuals with MND.
The dyadic nature of chronic illness implies that multiple aspects of one of the most important adult relationships, marriage, will be affected. Roles and responsibilities performed by each member of the couple will continually change as the disease progresses. The emotional trauma of adjusting to the unavoidable alteration in their relationship elicits strong emotions such as guilt, anger and frustration. Communication is the one of the most constructive ways of dealing with these emotions. The ability of spouses to convey their innermost thoughts, feelings and intimacy through communicative interaction is vitally important in marital communication.

Objectives: The aim of this study was to determine the association between the deteriorating speech of people with MND and the couple's perception of marital communication.

Methods: Fourteen couples (n = 28) divided into two participant groups, people with MND and spouses, participated in this non-experimental correlational research study. Data were collected during three visits at six-monthly intervals over a 12 month period. At each of these visits both participant groups completed a variety of objective and subjective measures, of which 20% were inter-rated by independent raters.

Results: Results confirmed the inevitable decline in speech intelligibility of people with MND across the disease progression. The people with MND did not report a change in their perception of marital communication although their spouses indicated a statistically significant decrease between the first and last visits. Interestingly, there was not a statistically significant relationship between the deteriorating speech of people with MND and the couples' perception of marital communication.

Conclusions: It is evident from this study that marital communication is not influenced by the deteriorating speech of people with MND. Even in the face of loss of speech, communication as a means of facilitating and maintaining the marital relationship is particularly pertinent to people with MND and their spouses. Health professionals should therefore take a holistic approach to provide timely and appropriate communication interventions that not only address the communication needs of the individual with MND but also consider the needs of their spouse to ensure successful intimate communication for the couple.

P288 ATTITUDES TOWARD LIFE SUSTAINING TREATMENT IN ALS-PATIENTS

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Keywords: life sustaining treatment, education, decision process

Background: In Germany there is no information about the frequency of invasive ventilatory treatment for ALS patients. Neurological centers mention percentages between 5%–7%. It is known that despite the physical impairment, the patients adapt to the progressing paralysis and experience a high quality of life. However, there is no information about how attitudes toward life sustaining treatment (LST) change in relation to the process of adaptation.

Objectives: The goal of the present explorative study was to measure patients' attitudes toward life sustaining treatment and toward end-of-life (EOL) issues.

Methods: The newly developed Life Sustaining Treatment Questionnaire (LSTQ) was applied. It comprised 115 descriptive items, distributed across attitudes toward four topics: (1) artificial nutrition, (2) respiratory mask, (3) tracheal ventilation (tracheostomy), and (4) EOL issues like death and euthanasia. The first three topics also included questions about the stability of attitudes; what kind of, and whether, decisions have been made; the reasons and certainty of these decisions; the information received; the persuasion experienced, and feeling of being a burden. Response options were either dichotomic, or on a Likert-scale (4 grades), multiple choice or open.

Results: Fifty-four ALS-patients were interviewed in their homes. Interviews lasted anything between one and two hours dependent on the patient's physical condition. Forty-four percent received sufficient information on artificial nutrition, 6% were persuaded to agree to this treatment, 24% made a decision for the treatment, 56% have not yet made a decision and 95% who had made a decision felt certain about it. Sixty percent of the patients received sufficient information about non-invasive artificial ventilation, 6% were persuaded to agree to this treatment, 57% decided for the treatment, 39% had not made a decision yet and 93% who had made a decision felt certain about it. Twenty-five percent received sufficient information about invasive ventilation, 5% were persuaded to agree to this treatment, 8% decided for the treatment, 60% had not made a decision yet and 84% who had made a decision felt certain about it.

Discussion and Conclusion: The acceptance of the LSTQ was high. The patients felt that questions were accurately verbalized and encouraged them to reflect about the disease. 8% refused the EOL questions because of emotional stress. In conclusion, ALS-patients received insufficient information about LST particularly with regards to invasive ventilation. Better information may reduce uncertainty and support

coping. Further, mostly the decisions were not influenced by others. The majority of those who had made a decision felt certain about it which underlines the notion that the decision for LST is a good predictor for coping with a life dependent on sustaining treatment.

P289 ATTITUDES OF ALS PATIENTS WITH REGARDS TO END-OF-LIFE ISSUES

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Keywords: end-of-life, advance directive, hastened death

Background: ALS-patients are confronted with end-of life (EOL) issues because of the progress of the disease. However, to date there has not been any specific information about how these people experience this process emotionally and cognitively.

Objectives: The goal of the current study was to measure attitudes of ALS patients with regard to EOL issues. Furthermore, the study intended to find those aspects which are the basis of these attitudes.

Methods: Thirty-one ALS-patients participated in the study. The following measures were administered: socio-demographical and disease related data, wish to hasten death (WTHD) by the "Schedule towards Hastened Death" (SAHD), quality of life by the "Schedule for the Evaluation of Individual Quality of Life" (SEIQoL-DW) and EOL issues by the newly developed "Life Sustaining Treatment Questionnaire" (LSTQ; Häcker, 2008, unpublished).

Results: Average SEIQoL scores were in the range of non-terminal chronic diseases ($M = 67.47 \pm 12.26$). The sample was split into two groups by using the criterion WTHD existent (WTHD = SAHD cumulative value of the patient > mean of SAHD + 1 standard deviation) or non-existent. There were no differences between these two groups with regards to their socio-demographical or disease related data as well as their quality of life. However, patients with WTHD mentioned "Autonomy", measured by the SEIQoL-DW, significantly more often as important for their QoL than those without WTHD ($Chi^2 = 6.62$, $p = 0.03$). Fifty-one per cent stated that they experienced fear of death. Those who were afraid of dying showed a higher fear of suffocating ($Chi^2 = 44.05$; $p = 0.00$) than the rest of the patients. Twenty-nine per cent of the patients had advance directives (AD). Sixty-four per cent of those patients without AD explained that they trust in significant others' abilities to represent their will. Fifty-five per cent stated that they were unable to make at this point in time the right decision with regards to the future. The approval of active euthanasia was correlated to the frequency ($r = 0.37$; $p < 0.05$) and intensity ($r = 0.31$; $p = 0.07$) of pain.

Discussion and Conclusions: Elucidating what determines patients' decisions toward the end life will help to provide optimally adapted palliative care. Fear of pain was prevalent among ALS patients and may have been a reason for the positive attitude toward legalizing assisted suicide. Likewise, ALS patients were afraid of suffocation. This demonstrated an alarming lack of information about available treatments. Patients have to be provided with all possible information and treatment to keep anxiety as low as possible, because anxiety seems to lead patients to wish for a hastened death. We argue that instead of legalizing assisted suicide we have to improve information transfer, palliative and terminal care.

P290 END-OF-LIFE DECISIONS AND ADVANCE CARE DIRECTIVES IN MOTOR NEURONE DISEASE: EVIDENCE BASED GUIDELINES FOR BEST PRACTICE

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Keywords: advance care directives, end-of-life decisions, autonomy

Background: The clinical management of ALS/MND is palliative from the time of diagnosis, and is focused on symptom control and adjustment to the progressive loss of neurological function with the certainty of early death. Formal guidelines regarding advance care planning, end-of-life decisions and advance care directives in neurodegenerative disease are currently unavailable in Ireland.

Objectives: 1) To identify the attitudes, understandings and experiences of patients with ALS/MND, their carers and their health-care providers to end-of-life decisions and advance care directives. 2) To determine if the views of patients, carers and their health-care providers concur. 3) To explore the relationship and impact of self reported quality of life, on patients with ALS/MND concerning end-of-life decisions and advance care directives. 4) To make recommendations for the formulation of advance care directives and end-of-life decisions in the ALS/MND population in Ireland.

Methods: Hermeneutic Phenomenology was chosen as an appropriate methodology for this study as it illuminates the meaning and understanding of the lived experience of contemplating end-of-life decisions and advance care directives for patients with ALS/MND, their carers and their healthcare professionals. Purposive sampling was used to identify participants for this study. Participants included patients, their carers, consultant neurologists, palliative care consultants and nurses. All participants had experience pertaining to the phenomena under investigation.

Results: This project has provided valuable insight into the attitudes, understandings and experiences of Irish patients with ALS/MND, their carers and their health-care professionals towards end-of life decisions and advance care directives. It has identified areas where attitudes of patients, carers and health professionals are not concordant. The project has identified a number of evidence-based guidelines that are pertinent to the formulation of advance care directives and advance care planning, within the confines of the Irish medical and legal system, and may be applicable to patients with other terminal conditions.

Conclusions: There are many ethical dilemmas to be considered in caring for patients with ALS/MND, especially regarding nutrition and respiration. It is essential that these issues are discussed in a timely and appropriate manner. Increasing our understanding of the attitudes, experiences and difficulties for patients, their carers and their health-care professionals in discussing end-of-life issues will ultimately improve practice. It is imperative that we avoid a "tick box" scenario to end-of-life management; individualised advance care plans need to be devised. As healthcare providers we have an obligation to create an appropriate atmosphere that encourages patients and their carers to discuss their legitimate

issues and fears regarding end-of-life decisions and advance care directives.

P291 PERSONAL ORIENTATIONS TO LIVING WITH ALS/MND: FINDINGS FROM THE STUDY OF UNSOLICITED WRITTEN ILLNESS NARRATIVES

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Keywords: coping strategy, illness narratives, personal experiences

Background: Health professionals are increasingly attempting to understand illness from the patient's perspective. Making use of illness narratives is one way of becoming more informed about the personal experience of living with illness.

Objectives: To explore the personal experience of living with ALS/MND as documented in personal illness narratives, written by people diagnosed with the illness.

Methods: We previously reported the processes used for locating published and unpublished personal illness narratives about life with ALS/MND (1). The 161 narratives identified were subject to content and thematic analysis. Data management was aided by Nvivo 7 software. A number of themes were identified from the analysis.

Results: We focus here on the theme 'personal orientation to illness' which is concerned with the varying approaches adopted by people with ALS/MND to manage both the physical and psychological impact of their condition. Authors write of modification as they adapt to the effects of their disability. There is tolerance in the acceptance of illness, often emerging from a period of intense turmoil. A positive attitude is emphasised in reflections on what can be done, rather than dwelling on what has been lost. The search for a cure pervades the narratives, whether through conventional, experimental or alternative treatments. Remarkably, many authors identify benefits to their diagnosis.

Conclusions: Here we have accounts that demonstrate how individuals with ALS/MND adapt to their illness; their personal strategy for coping. A variety of approaches are advocated as people undergo the transition from health to ill health. What emerges for many is a sense of bringing priorities into clearer focus and putting irrelevancies to one side. Written illness narratives provide understanding of the experience of illness; by familiarising themselves with them, those caring for the sick and dying can 'learn how medicine looks from the patient's point of view' (2). Stories of living with ALS/MND are regarded as excellent teaching tools, providing insight not obtainable from classic textbook descriptions (3).

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