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SESSION 1 JOINT OPENING SESSION

C1 ALS: ONE DISEASE OR MANY?

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In spite of valiant effort, traditional pharmacotherapeutic interventions have yet to make a significant impact on the course of ALS. To patients and care-givers, as well as investigators, this is a great source of frustration. Here, we will examine the clinical and biological evidence that ALS cannot be a single disorder and that viewing it in such a framework allows for a novel approach to pharmacological strategies.

The first *clinical evidence* is phenotypic and centres on the both anecdotal and evidence-based observations of extreme variability in survivorship. While site of onset, age at symptom onset, and the initial rate of progression impact on traditional survivorship curves, there remains a significant subgroup of ALS patients with exceptionally long survivorship. Conversely, a more malignant variant of ALS exists with survivorship less than a year. A major advance in our understanding of ALS has been the recognition that the biological process of ALS can also impact on cognition, behaviour or executive function. This is more than a curiosity in that such non-motor involvement impacts on survivorship. The challenge is to determine whether subtle syndromes of frontotemporal dysfunction have the same impact.

The *biological evidence* that ALS is a heterogeneous disorder is overwhelming. The genetic evidence, perhaps the most convincing, will be dealt with in detail by my colleague Dr Andersen. Although the struggle for survival of the motor neuron in ALS is a delicate balance between a host of both neuronal and non-neuronal cells, few investigators anticipated the extreme complexity of this interaction. The activation of microglia, long thought to be the harbinger of neuronal death, clearly may be neuroprotective. Astrocytes, the seemingly quiescent bystander in the neuronal neighbourhood, contribute to the maintenance of neuronal health but may fail dramatically in ALS. However, unravelling the metabolic turmoil of the motor neuron in ALS is perhaps our greatest advance. Once thought to be independent processes, it is now clear that there is a tight inter-relationship amongst the biochemical disturbances of the motor neuron, including excitotoxicity, intermediate filament metabolism, oxidative injury and inflammatory cascades. Alterations in RNA stability may provide a unifying mechanism for both sporadic and familial ALS through the discovery that TDP-43 is a messenger RNA binding protein that forms a complex with copper/zinc superoxide dismutase (SOD1), 14-3-3 protein and neurofilament mRNA. The observation of both tau protein mutations and altered phosphorylation state in ALS in the presence of a frontotemporal syndrome brings the biochemistry and clinical evidence of heterogeneity full circle.

Conclusion: There is little doubt that ALS is clinically and biologically a heterogeneous disorder. However, embracing this knowledge has opened tremendous opportunities to selectively target specific aspects of the disease process for disease process modifying interventions and the promise of great advances in our treatment repertoire.

C2 THE GENETICS OF ALS/MND AND THE ROLE OF MODIFIER GENES: A CLINICAL PERSPECTIVE

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Though most ALS patients do not have a close relative affected with ALS, 5–13% of patients report having a relative with ALS (familial ALS, FALS). Genetic and genealogical studies have revealed that this figure may be too low. The nature of the disease has made it difficult to collect large numbers of blood samples from many affected patients to find the genes involved. Despite intensive research around the world, only five genes and eight possible genetic loci have been identified.

The five genes are SOD1, Alsin, Dynactin, SETX and VAPB. At present, mutations in the latter four genes appear to be very rare and analysis is only performed in a scientific setting. A few patients (usually diagnosed as sporadic ALS (SALS)) with private mutations in the NF-H, EAAT2, NAIP, angiogenin, peripherin, HFE, PON1, PON2 and SPG4 genes have also been reported but causation remains to be proved.

Since 1993, 139 mutations have been found in the SOD1 gene on chromosome 21 with five different modes of inheritance: dominant inheritance with high penetrance, dominant inheritance with reduced penetrance, recessive inheritance, compound heterozygosity and a de novo mutation. The most frequent SOD1 gene mutation is the D90A which in many European countries is inherited as a recessive trait with a characteristic slowly progressing phenotype though pedigrees with dominantly inherited D90A-SOD1 and an aggressive phenotype have also been reported. The most frequent mutation in North America is the A4V which is associated with a very aggressive form of ALS. In different populations, 12% to 23% of patients diagnosed with FALS and 2 to 7% of SALS patients carry a SOD1 mutation. Diminished disease penetrance is not infrequent for carriers of some SOD1 gene mutations (like the I113T, G93S, D76Y) and SOD1 mutations can be found in cases of apparently SALS. There is no specific therapy for patients with a SOD1 gene mutation but a number of projects are underway to develop techniques (RNAi, antisense therapy) to inactivate the mutant SOD1 gene to prevent the synthesis of the cytotoxic mutant SOD1 protein. However, finding a patient to be a carrier of a SOD1 gene mutation can be of advantage to the

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patient. A DNA-SOD1 test speeds up the diagnostic process and can be of help in patients with atypical features as well as providing prognostic information. Genetic counselling can be better provided to the patient and relatives. Presymptomatic (predictive) genetic testing should only be performed in first-degree adult blood-relatives of patients with a known SOD1 gene mutation. Testing should only be performed on a strictly volunteer basis. Special consideration should be taken before presymptomatic testing is performed in families where the mutation is associated with reduced disease penetrance or with a variable prognosis.

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SESSION 2A NEUROGENOMICS

C3 MAKING THE MOST OF YOUR MICROARRAY

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DNA microarrays can perform whole transcriptome assessment in a single experiment. However, the analysis of microarray data is challenging, and the obtained dataset usually contains both *type I* and *type II* errors. As a result, validation of the findings with an independent method is strongly recommended. This is especially important in brain transcriptome profiling experiments, where the magnitude of the mRNA expression change at the tissue level most often does not exceed 50%. Technical replicates of a microarray dataset, starting with new cDNA synthesis from the same RNA, are very helpful for estimation of assay noise and false discovery rate (FDR). For identification of differentially expressed genes, implementing dual statistical criteria, based on both magnitude of change and probability of change, coupled with a permutation analysis of the data can usually uncover the critical expression differences while keeping FDR at a low level. However, regardless of the analysis performed, negative data should be cautiously interpreted as microarrays often do not detect all real, biologically important expression differences.

Once the differentially expressed genes are identified, the data analysis moves to a pattern mining phase. In the first step genes are grouped together based on structure, function or common motifs in the DNA sequence. In the second step, the dataset is assessed for differentially expressed genes that show common structural or functional characteristics that reach beyond what would be expected by chance. These analyses often uncover altered molecular pathways or co-regulatory patterns that are not obvious at the single-gene analysis level.

The true power of these approaches can be best demonstrated using DNA microarray studies with a complex, converging design. For example, to determine presenilin-1 (PS1) regulated genes, first we compared the neocortical and hippocampal transcriptome of PS1 conditional KO mice to those of wild-type littermates. Next, we compared the transcriptomes of transgenic mice carrying the wild-type human PS1 to that of transgenic mice carrying the Familial Alzheimer Disease-linked delE9 mutant human PS1. Cross-correlating findings revealed a number of transcripts showing differential expression across these two datasets. These expression changes, involving many early-immediate gene (IEG) transcripts, were also regulated in the amyloid-depositing APPswexdelE9PS1 mutant mice and showed a strong pattern reversal when the same mice were subjected to environmental enrichment. The overall data, obtained across five different animal models, suggest that PS1 is a potent regulator of IEG expression in an amyloid-dependent fashion and that environmental enrichment prevents amyloid accumulation in the brain tissue through a mechanism closely linked to the IEG transcript network. Finally, based on recently obtained cortical transcriptome data in brain-derived neurotrophic factor (BDNF) KO mice, we speculate that the PS1-amyloid system in the brain tissue is strongly influenced by BDNF-mediated molecular cascades.

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C4 GENE EXPRESSION PROFILING TO ELUCIDATE PATHWAYS OF MOTOR NEURON INJURY IN ALS/MND: A REVIEW OF THE CURRENT POSITION AND FUTURE POTENTIAL

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Background: Despite intensive research effort, the causes of motor neuron injury in ALS/MND remain incompletely understood, even in the genetic subgroup caused by SOD1 mutations. In the last 5 years microarray analysis has been applied to the problem of motor neuron degeneration. Early studies tended to employ tissue analysis of whole spinal cord, in which changes in the motor neuron transcriptome are likely to be masked by changes in other cell types. Recently, laser capture microdissection, combined with microarray analysis, has allowed the identification of gene expression changes in specific cell types.

Objective: The initial aims of the functional genomics programme at the University of Sheffield have been to: 1. To identify the cell specific properties of motor neurons and differences in gene expression between groups of MN vulnerable and spared in the disease process in ALS; 2. To understand the cellular pathways of motor neuron injury in the presence of mutant SOD1 at different time points; 3. To identify targets for drug therapies.

Methods: We have used a combination of a cellular model (NSC34 cell line stably transfected to express mutant or normal human SOD1) and the G93A mSOD1 mouse model. Important changes are checked in relation to human ALS using CNS tissue from patients (Sheffield Brain Tissue Bank). Motor neurons are isolated from spinal cord sections using the Arcturus Pixcell laser capture microdissector. RNA is extracted using Picopure kit (Arcturus), amplified (where necessary) using the RiboAmp Amplification kit (Arcturus) and labelled using the BioArray High Yield RNA Transcript Labelling Kit (Enzo). 10μg cRNA is applied to the relevant Affy mouse, rat or human GeneChip, and data analysis is performed using ArrayAssist System (Iobion); Pathway Architect and GenMAPP1.1.

Results: The application of microarray technology to models of ALS and the human disease will be reviewed. In addition new insights arising from 3 key studies will be discussed and illustrated:

1. Transcriptome changes in the NSC34 cell line in the presence of mutant SOD1. 268 genes were differentially expressed and key insights were the overall transcriptional repression and the down-regulation of the Nrf2 anti-oxidant response element genes. The development of this pathway as a target for neuroprotective therapy with Nrf2 inducing drugs will be illustrated.
2. Gene expression changes in motor neurons at 3 disease time points in the G93A mouse model have been investigated. Key changes at the pre-symptomatic stage (252 genes differentially expressed) include a marked upregulation of lipid and carbohydrate metabolism and mitochondrial function, as well as genes involved in transcription and translational functions. At the late disease stage (120days) 167 genes are significantly altered, with the development of marked transcriptional repression, but up-regulation of complement system components and key cyclins involved in cell cycle regulation.
3. The gene signature of normal motor neurons, compared to other neuronal groups (sensory neurons and Purkinje cells) has been investigated in the rat. 89 genes were expressed at significantly higher levels in motor neurons including members of 8 important gene categories eg genes involved in myelination, energy metabolism, cytoskeletal and transport components and calcium regulation.

Conclusions: This overview will illustrate the potential of microarray technology to understand in greater depth the cell specific features of motor neurons and the cellular pathways which become dysfunctional during motor neuron injury, which potentially represent targets for neuroprotective therapy development.

C5 COMBINED OLIGONUCLEOTIDE AND TISSUE MICROARRAYS TO ASSESS CELL-TYPE SPECIFIC FACTORS CONTRIBUTING TO NEURODEGENERATION IN ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease that specifically affects motor neurons in the central nervous system (CNS). The mechanisms that lead to selective motor neuron death remain unknown. Recently, glial cells have been implicated in motor neuron degeneration.

Objectives: To identify common features of motor neuron cells and, separately, glial cells that participate in motor neuron degeneration. We examined gene and protein expression profiles using array techniques in two mouse models of ALS: familial ALS linked to SOD1 (G93A) and frontotemporal dementia with ALS linked to mutant TAU (P301L).

Methods: Lumbar spinal cords from 3 month old female transgenic mice and their non-transgenic littermates were dissected and cryosectioned axially. Sectioned slides were fixed in ethanol and stained with Cresyl violet. In order to complement our study performed on enriched motor neurons from mouse models of motor neuron degeneration (1), we have collected glia surrounding motor neurons from the anterior horn of the spinal cord, and examined their gene expression profiles. Glial cells were laser-capture microdissected using PixCell Arcturus. RNA was extracted from these cells and underwent microarray experiments using Aglilent's Mouse Whole Genome Oligonucleotide Microarray. After statistical analysis of the differentially expressed genes, we have further investigated the relevance of our findings with respect to human disease by performing protein expression analysis on post-mortem ALS samples using tissue microarray technology (TMA). We have constructed ALS TMA consisting of CNS tissues from ALS patients, ALS with frontotemoporal dementia (FTD) patients, and age and sex matched controls. Each TMA block includes ALS, ALS with FTD, and control samples containing the most affected regions, such as the cervical and lumbar spinal cord, as well as less vulnerable regions, such as the cortical and subcortical areas of the brain.

Results: We have identified genes with expression profiles shared by the two models. We have identified 8 genes with similar expression patterns in the motor neurons of the two mouse models. Of these, 5 commercially available antibodies against proteins for which they encode were tested on our TMA showing distinct patterns in motor neurons and glia.

Discussion and conclusions: This is the first global gene expression study that uses enriched populations of both cell types thought to be involved in ALS, motor neurons and glia, to identify cellular factors specific for each cell type. The study is not limited to the evaluation of animal models of ALS, but confirms the findings in human material thus validating the relevance of our findings for ALS. We are planning to further follow up on the identified cellular factors and pathways to identify new therapeutic targets.

Reference:

1. Kudo *et al*, Amyotrophic Lateral Sclerosis 2006 (Suppl 1); 7

C6 DIFFERENTIAL GENE EXPRESSION IN PERIPHERAL BLOOD OF ALS PATIENTS ASSOCIATED WITH GENETIC VARIATION

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Background: The genetics of the sporadic form of Amyotrophic Lateral Sclerosis is still largely unknown. Combining the genetical variation with genome wide gene expression profiles of complete blood (genetical genomics) within one individual will give insight into genes associated with the disease.
As for diagnosis or disease progression in ALS, neither definitive diagnostic tests nor surrogate markers are available. Recently proteomic profiling of liquor by mass spectrometry identified protein species with altered levels in ALS patients. It is hypothesised that blood genomic fingerprinting may be a way to find candidate markers.

Objective: By using expression profiling, to look at the possibility of using blood as a surrogate tissue in the diagnostic phase and look for candidate genes involving pathogenesis and disease progression.

Method & Results: In 116 ALS patients and110 matched healthy controls whole blood gene expression profiling using Illumina Sentrix HumanRef-8 Expression BeadChip was combined with genome wide genotyping using 300K Illumina Infinium BeadChip.
By dividing the samples into two datasets, one group can serve as a validation dataset. Using weighted gene co-expression network analysis (WGCNA) genes can be grouped into modules with similar expression patterns. In the first dataset two out of the seven identified modules were differentially expressed in ALS patients and validated in the second dataset. Mean expression per module (principal component) was mapped on the genome treating it as a quantitative trait. SNPs associated with the differential expressed modules are significantly associated with ALS status.

Conclusion: We found differentially expressed genes in peripheral blood that can be validated in a second dataset. These genes can be used as a biomarker for ALS. Combined analysis with genomic information gives more insight into pathways involved in ALS.

C7 DIFFERENTIAL GENE EXPRESSION IN SALS MOTOR NEURON AND ANTERIOR HORN ENRICHED RNA POOLS USING WHOLE-GENOME OLIGONUCLEOTIDE MICROARRAY

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Background: New whole genome microarray technologies provide comprehensive and unbiased profiles of gene expression. Applied to SALS, major challenges include the highly selective nature of degeneration, the highly variable rostral-caudal distribution of degeneration, and the potential for degradation from necrolysis.

Objectives: To profile differential gene expression in the cellular compartments relevant to motor neuron degeneration in SALS.

Methods: We created a tissue bank of SALS nervous systems specifically for molecular investigations. In each nervous system, we profiled molecular quality and pathological burden and based on this, we selected nervous systems for further evaluation. From these, we created 2 separate RNA pools, one enriched with motor neurons isolated by laser capture microdissection (LCM) and one enriched with anterior horns collected after removal of motor neurons by LCM. We synthesized cRNA probe using 2 cycles of reverse transcription and *in vitro* transcription amplification. We profiled gene expression using whole genome oligonucleotide microarray. We verified molecular quality at each step. We processed microarray data with GCOS (Gene Chip® Operating Software from Affymetrix), GC-RMA transformation, and 2-way ANOVA. We scored biological processes using DAVID and Gene Ontology (www.david.abcc.ncifcrf.gov.org and www.geneontology.org).

Results: We profiled 8 controls (mean age 62 years) and 9 SALS (mean age 68 years). All SALS patients had bulbar or arm onset disease and a descending degeneration with moderate preservation of lumbar motor neurons, which is where profiling was performed. Average percent positive calls on the microarrays were 42% (35–46%) for controls and 44% (39–48%) for SALS—by comparison, our average percent positive calls in comparable studies on mice was 50%. 1665 of ~39,000 (4.3%) possible transcripts were differentially expressed with $p < 0.05$; 1287 (77%) were up-regulated and 378 (23%) were down-regulated. Up- and down-regulation seemed essentially parallel and commensurate in motor neurons and anterior horns enriched pools. Among the most enriched biological processes were neuron maturation, development and differentiation; nerve ensheathment; neurogenesis; ionic insulation of neurons by glial cells; and metabolism. Among the most down-regulated biological processes were phosphorylation, protein transport, and RNA splicing. Genes involved with TDP-43, ubiquitin, inflammation, apoptosis, and cytoskeletal regulation were differentially

expressed but this was modest in relative terms. (All data is undergoing significant refinement.)

Discussion and conclusions: We profiled differential gene expressions in motor neuron and anterior horn enriched pools in lumbar spinal cords of SALS. The quality of the expression profiles using our paradigm approaches controlled experimental conditions. Because of the topographic distribution of pathology in our series, our profile is weighted toward early degeneration and upstream pathways. We did not find obvious differences in up- or down-regulation of genes between the motor neuron and anterior horn enriched pools. The challenge now is determining the biological significance—one promising strategy is to correlate genome-wide ALS-associated SNPs and our expression profiles and this is addressed in our companion abstract (C8).

C8 CORRELATIONS BETWEEN WHOLE GENOME SNP AND GENE EXPRESSION PROFILES: DISCOVERY-BASED PURSUIT OF SALS PATHOBIOLOGY

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Background: Genome-wide SNP association studies in SALS identify sites of interest in the genome by statistical methods but they lack direct biology. Whole genome expression studies, on the other hand, identify genes of interest directly in the biological tissues but interpretation is difficult. Since they are complementary data sets, together they have the potential to identify important aspects of pathobiology.

Objective: To correlate SNPs and differentially expressed genes in SALS.

Methods: We took tag SNPs of interest in SALS from the North American genome-wide genotype association study and identified SNPs associated with them by linkage disequilibrium (LD) (rel#21 NCBI B35) (www.HapMap.org). We associated the complete set of SNPs with genes (dbSNP build 123) (www.snpper.chip.org). We identified those that were differentially expressed and we annotated and scored their biological processes (www.geneontology.org and www.david.abcc.ncifcrf.gov.org).

Results: Of the 16,833 tag SNPs associated with SALS with $p < 0.05$, all were annotated and 7,004 were associated with ~3,170 genes. 85,442 SNPs were associated by LD ($r^2 > 0.75$) with these tag SNPs, 54,435 were annotated, and 20,396 were associated with ~2,841 genes, expanding the list of genes of interest by ~734 or 23% to a total of ~3,903. Approximately 6–7% of genes associated with SNPs were differentially expressed and this percent did not vary with p-value of the SNP association - ~230 of 3903 genes from SNPs with $p < 0.05$, ~71 of 1027 genes from SNPs with $p < 0.02$, ~31 of 527 genes from SNPs with $p < 0.01$, and ~14 of 215 genes from SNPs with $p < 0.005$ were differentially expressed. Differentially expressed genes from SNPs with $p < 0.01$ were BARD1, AKAP7, ZNF100, DPF3, CSPG2, WWOX, ZNF70, PRKCA, SUCLG2, MGC72075, NUP50, C20orf43, RORA, IVD, SGCD, RFX3, GLIS3, DKK3, ASTN2, SFRP2, PLEKHA5, PCNX, CRIM1, ZNF84, BMP2, MME, PKP4, CYP4F3, ERCC1, KCNMA1 and RNF126. The biological processes of these genes and their enrichment scores included: cellular metabolism, 2.98; cell communication, 1.66; cell organization and biogenesis, 1.75; RNA processing, 1.08; apoptosis, 0.65; DNA and metabolism, 0.58—the range of enrichment scores of biological processes of all differentially expressed genes was 0–2.98. (Both SNP and expression data are undergoing major refinements.)

Discussion and conclusions: 1) Correlating whole genome SNP and gene expression profiles together has potential to identify important pathobiology of SALS with a discovery-based approach. 2) LD may expand the genes of interest by as much as ~23%. 3) Strategies for interpretation are unsure: false positive and false negative errors are inevitable and increasing the degree of statistical association of the SNPs may not enrich the list of genes and biological pathways of interest. 4) The effectiveness of this approach will improve as tools and methods are refined. 5) Limitations and pitfalls of this approach include: causal SNPs may not necessarily cause differential gene expression; differential gene expression is as much a result as a cause of essential pathobiology; and there is no exploration of non-coding regions of the genome.

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SESSION 2B PALLIATIVE CARE

C9 WHOLE PERSON CARE IN ALS/MND

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This presentation is a tribute to the work of Balfour Mount, MD, one of the pioneers of Palliative Care. Dr. Mount founded the first acute care unit for the terminally ill worldwide in 1975 at the Royal Victoria Hospital, part of Montreal's McGill University. He coined the term ''Palliative Care'', which has since become a worldwide standard. Dr. Mount's work embraces all aspects of Palliative Care, with a particular focus on spiritual and existential aspects. He has always been particularly interested in ALS/MND, and has initiated a movie on the life of Phil Simmons, the author of the poignant ALS autobiography, ''Learning to Fall''. In 1999, Dr. Mount founded the Program for Whole Person Care at McGill University, which is devoted to spreading the principle of Whole Person Care throughout modern medicine.

What, then, is Whole Person Care? *''In situations in which treatment is unable to change the disease outcome, it may be possible to create a space in which healing can occur. Lessons about quality of life and individuation, learned in the arena of advanced illness, also have relevance earlier in the disease trajectory and for those who are physically well. While the existential/spiritual domain is known to be an important determinant of quality of life, there has been little emphasis on integration of the issues in health care''* (excerpted from www.mcgill.ca/wholepersoncare)

Research in ALS/MND has been pivotal in showing that quality of life (QoL) in severely ill patients is not primarily dependent on physical function, but rather on non-physical determinants such as hope, dignity, personal values, social relationships, meaning in life, and spirituality. Data from recent studies show how each of these concepts strongly influences subjective well-being and end-of-life decisions. The family plays a crucial role, both as the main factor influencing QoL and as part of the ''unit of care''. Since at present the main goal of care in an incurable disease such as ALS/MND is the maintenance of the highest possible QoL until death (which corresponds to the WHO definition of palliative care), a higher proportion of the available resources should be devoted to the development and implementation of interventions in these areas. We have recently developed a patient-generated instrument for the assessment of individual meaning in life (the Schedule for Meaning in Life Evaluation, SMiLE) which can also be used as the basis for a simple meaning-enhancing intervention in severely ill patients.

As Bal Mount says, ''Healing happens in the present moment''. Learning to live in the present moment is a gift that is most often imparted by our patients to us, not vice versa. Ultimately, the concept of Whole Person Care not only applies to the patients and their families, but to the professional caregivers as well.

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C10 END-OF-LIFE CARE AND DECISION MAKING IN ALS/MND: A CROSS-CULTURAL STUDY

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Background: The care of a person with ALS/MND is complex and as the disease progresses there are many decisions that need to be taken to ensure the quality of life is as good as possible. These include the decision on gastrostomy, ventilatory support and end of life care.

Objectives: This study aimed to look at these aspects of care in three countries – the UK, USA and Japan, considering the cultural differences from country to country.

Method: A questionnaire was sent to six hospices in the UK, a palliative care team in the US and a special interest neurology group in Japan and it was completed for at least the last 10 ALS/MND patients who had died. The questionnaire covered the details of the patient and the use and discussion of gastrostomy, ventilation and end of life care.

Results: One hundred and twelve questionnaires were returned (47 UK, 10 USA, 55 Japan). The patient groups were largely similar in age and gender mix but the duration of the disease was higher in Japan, as there was a greater use of ventilatory support – UK mean duration first symptom to death 30 months, USA 29 months and Japan 48 months. Eighty-six per cent of patients died from respiratory failure or pneumonia.

The use of interventions and discussion about end of life issues varied: Gastrostomy 23% (UK), 50% (USA), 35% (Japan); Noninvasive ventilation 13% (UK), 20% (USA), 36% (Japan); Invasive ventilation 4% (UK), 0 (USA), 9% (Japan); Discussion about gastrostomy 79% (UK), 70% (USA), not recorded (Japan); Advance directive discussion 23% (UK), 80% (USA), not recorded (Japan); Cognitive changes noted 26% (UK), 20% (USA), 7% (Japan).

Opioids were widely used, although the use varied between centres.

Conclusion: 1) There are differences from country to country and from unit to unit within a country in the assessment and management of ALS/MND. There are widespread discussions about the management of disease progression, although this varies between countries. 2) These discussions should occur early in the disease progression, as cognitive loss may subsequently alter the ability of the patient to be fully involved. 3) These differences will impact on patient care, as patients increasingly have contact with patients, families and professionals from other countries and cultures via the internet.

C11 MEDICAL AND SUPPORTIVE CARE AMONG PEOPLE WITH ALS IN THE MONTHS BEFORE DEATH OR TRACHEOSTOMY

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Background: The final months of life for people with ALS are challenging because of increasing disability. Also, families need to insure appropriate palliation and planning for the end of life, or reach clarity on decisions to undergo tracheostomy and long-term mechanical ventilation. Little information is available on service use and decision-making at the end of life among people with ALS.

Objectives: In monthly interviews with family caregivers and patients approaching pulmonary failure, we examined (i) the prevalence and timing of 17 different end-of-life decisions, ranging from use of augmentative communication devices to designation of health care proxies; and (ii) differences in end-of-life decisions between patients opting for tracheostomy and patients who died.

Methods: Eighty patients with forced vital capacity (FVC) $<50\%$ and their family caregivers were enrolled from an American ALS tertiary care center between 2000–2004. Personal interviews were conducted monthly. We examined the proportion of patients using each end-of-life option, its timing relative to death or tracheostomy, and whether patients using tracheostomy were more or less likely to use the different options.

Results: The median age at study entry was 62. Fifty-six per cent of patients were male and 85% white. All but 10% completed high school. Of the 80 patients, 16 (21.1%) opted for tracheostomy and long-term mechanical ventilation, of which two died during or shortly after the procedure. Four patients were alive or lost to follow-up. The remaining 60 (78.9%) died during follow-up. Patients opting for tracheostomy were more likely to use supportive and medical care options before the event: 87.5% vs. 41.7% for psychological counselling ($p=0.001$), 87.5% vs. 55.2% for psychiatric medicine ($p=0.02$), 68.8% vs. 30% for complementary medicine ($p=0.005$), 68.8% vs. 40.7% for PEG placement ($p=0.05$), 93.8% vs. 64.4% for nasal ventilation ($p=0.02$), 87.5% vs. 50% for paid personal assistance care ($p=0.007$), 75% vs. 39% for use of the emergency room ($p=0.02$), and 62.5% vs. 30.5% for hospital admissions ($p=0.01$). Patients who died and patients who opted for tracheostomy did not significantly differ in autopsy consent, use of health care proxies or power of attorney, or participation in clinical trials. Use of tracheostomy was associated with less use of hospice (31.3% vs. 72.1%, $p=0.002$), but 5 of the 16 tracheostomy patients used hospice at some point before the procedure.

Discussion and conclusions: The choice to use long-term mechanical ventilation is part of a broader pattern of intensive service use designed to prolong life in the face of impending pulmonary failure. About a third of patients choosing tracheostomy make the decision relatively late in the course of the disease and remove themselves from hospice care.

C12 A 20 YEAR STUDY ON OUTCOMES OF ALS/MND PATIENTS USING TRACHEOSTOMY VENTILATION: WHAT EVERYONE NEEDS TO KNOW

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Background: ALS/MND invariably results in respiratory failure, unless mechanical ventilation (MV) is used. Tracheostomy positive pressure ventilation (TPPV) often follows emergency hospitalization and uninformed decision-making. Noninvasive positive pressure ventilation (NPPV) has become the treatment of choice for ALS/MND. Immobile patients do not often return to clinics for pulmonary evaluation or optimal use of NPPV.

Objectives: The purpose of the study was to determine: 1. Factors contributing to unexpected respiratory failure, emergency hospitalizations and commencement of TPPV; 2. If use of NPPV prevented unplanned TPPV; 3. Respiratory status prior to TPPV; 4. What patients believed about TPPV; 5. The occurrence of severe immobility and locked-in state; 6. Burden of care factors; 7. The causes of deaths of TPPV users; 8. Outcomes of MV users with ALS/MND.

Methods: Patients with ALS/MND were referred for nursing consultation on management of care. Data were collected from visits to homes or care facilities; periodic phone calls; questionnaires and interviews with caregivers.

Results: One hundred and eighteen patients with ALS/MND commenced TPPV during acute respiratory failure (ARF) and emergency hospitalization. One hundred and five (89%) of 118 did not plan TPPV ahead. Thirteen (11%), who planned TPPV in advance, began TPPV during unexpected ARF. Twenty-one (18%) of the 118 were previous NPPV users, including 4 who used NPPV

for a mean of 35 months. When they chose TPPV, 50 (42%) of the 118 were ambulatory, and indicated they were not ready to die. Thirty (25%) engaged in strenuous activities when ARF occurred. Thirty-four (29%) began TPPV within 3 months of the ALS/MND diagnosis. Thirty-eight (32%) had prior pneumonia. Ten had unexpected ARF after PEG tube placement. Sixteen patients, who began emergency TPPV, were previously denied NPPV because their pulmonary tests "looked good," despite orthopnea and use of accessory muscles. Twenty-five (21%) of 118 had no previous pulmonary evaluation. Twelve (10%) began TPPV, after failed use of NPPV during ARF. Twenty-one NPPV users had no pulmonary evaluation or NPPV management prior to emergency TPPV. Of the 21, 10 became intolerant of NPPV due to severe bulbar impairment. Five had ARF, while off NPPV, and began TPPV. Seventy-eight (66%) of 118 believed TPPV would be short term and that progressive immobility predicted death. Thirty (25%) of 118 were waiting for a cure and reversal of paralysis. Eighty-two (69%) of 118 became quadriplegic within five years. Eighteen became totally locked-in. Slow communication and immobility, not tracheostomy care, caused highest burden of care. Thirty-four (29%) of 118 withdrew from TPPV. Twenty-three (20%) died from cardiac arrest.

Conclusion: Unplanned TPPV occurred despite NPPV use. Factors triggering respiratory failure and unplanned TPPV: overexertion, unawareness of pending ARF, overlooking respiratory signs and test results; prior pneumonia, NPPV intolerance, failed or inadequate use, management of NPPV; and PEG tube placement. Ambulatory and respiratory onset patients are risks for unplanned TPPV. Everyone needs to know: TPPV is not a short-term treatment, immobility will progress and is not a predictor of death, and treatment to reverse paralysis is unlikely. Patients have the right to withdraw treatment.

C13 DEVELOPING A NATIONAL END-OF-LIFE CARE STRATEGY: THE CANADIAN EXPERIENCE

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Over the last six years Canada has worked on developing a Hospice Palliative End-of-Life Care Strategy that would engage all levels of government, the health care provider community, patients, families and caregivers. There have been many successes and a few failures along the way but we are working towards an integrated, comprehensive and coordinated approach to end of life care.

This presentation will include some background around the need for this framework, and what has been achieved or attempted towards this goal in Canada including our advocacy and policy development strategies.

As well, this presentation will set out some of the future challenges in order to achieve a coordinated national end of life care strategy/framework to meet the increasing needs as our population ages.

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SESSION 3A SELECTIVE VULNERABILITY OF MOTOR NEURONS

C14 MITOCHONDRIAL MUTATIONS AND SELECTIVE NEURONAL VULNERABILITY: LESSONS FOR ALS

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Abstract not available.

C15 CONTROL OF NEUROFILAMENT EXPRESSION IN MOTOR NEURONS: ROLES IN DEVELOPMENT AND DISEASE

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Background: Neurofilaments (NFs) are the most abundant cytoskeletal component of motor axons. They are made of NF subunit proteins whose expression levels must be tightly coordinated to maintain neuronal homeostasis; imbalances in this expression can lead to aggregation of NFs, a hallmark of ALS. NF expression is controlled not only transcriptionally, but also post-transcriptionally. Recent studies have implicated aberrant post-transcriptional regulation as a likely contributing factor to the neurodegenerative disease process.

Objective: To better understand the post-transcriptional control of NF expression and its role in maintaining neuronal homeostasis, we are studying both its contributions to NF expression and the individual ribonucleoproteins (RNPs) involved during development and in meeting traumatic challenges to the CNS.

Methods: We used the following: 1) Affinity purification from rat brain followed by mass spectrometry to identify RNPs that bind the middle NF subunit's RNA (NF-M); 2) Co-immunoprecipitation assays to quantify endogenous binding of RNPs to NF subunit RNAs during development; 3) RNA-footprinting to map where these RNPs bind along the NF-M RNA; 4) Polysomal profiling, quantification of intracellular RNA pools, and *in vitro* RNA decay assays to characterize the contributions made by RNA translation, transport, and stability to NF-M expression during optic nerve regeneration in frog (*Xenopus laevis*); 5) Targeted gene knockdown in frog embryos by antisense morpholino oligonucleotides to assay the role of individual RNPs during motor axon development.

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Results: In rat, we identified three RNPs (hnRNPs K, E1, and E2) capable of binding NF-M RNA. These proteins bound all three NF subunit RNAs endogenously, and their binding was developmentally regulated. RNA-protein interactions occurred in three separate domains along the NF-M RNA, each of which bound the various RNPs differentially, suggesting these interactions play separate roles under differing circumstances. To better understand how neurons use post-transcriptional control of NF expression to successfully meet traumatic challenges, we studied NF-M RNA during frog optic nerve regeneration. The most dramatic changes correlating with regenerative axonal outgrowth occurred in nucleocytoplasmic RNA transport and in translational efficiency. Targeted gene knockdown experiments in *Xenopus* embryos revealed that hnRNP K is essential for NF-M RNA to undergo both these processes during motor axon development.

Discussion and conclusions: These results support the idea that NF expression is under significant post-transcriptional control that is central to normal development and in responding successfully to injury. This conclusion raises the hypothesis that variations in this control among neuronal populations may contribute to their differing susceptibilities to neurodegenerative disease.

Acknowledgements: NSF grant IOB 0643147 supports this work.

C16 DISRUPTION OF ASSEMBLY AND AGGREGATION OF LIGHT NEUROFILAMENT (NFL) PROTEIN IS A COMMON TRIGGERING EVENT IN MOTOR NEURON DISEASE

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Background: Mutations in light neurofilament (NFL) and small heat shock proteins B1 (HSPB1) and B8 (HSPB8) cause autosomal dominant axonal Charcot-Marie-Tooth disease type 2E (CMT2E), 2F (CMT2F) and 2L (CMT2L). Previous studies have shown that CMT mutations in NFL (1,2), HSPB1 (3) and HSPB8 (4) disrupt assembly and cause aggregation of NFL protein.

Objectives: To determine the role of disrupted assembly and aggregation of NFL protein in mediating neurotoxic effects of different disease-causing mutations in motor neurons.

Methods: We examined the effects of expressing wild-type or disease-causing mutant NFL or HSPB1 in primary cultures of mouse motor neurons using co-expression of a GFP reporter transgene or a GFP-tagged NFL protein to monitor live targeted motor neurons by fluorescent and phase-contrast microscopy for up to a week following microinjection of wild-type or mutant cDNAs directly into motor neurons. Alterations in live neurons were correlated with changes in the disposition of anti-NFL and anti-HSPB1 immunoreactivities in targeted neurons.

Results: Whereas expression of wild-type human NFL becomes widely disposed in filamentous array throughout the perikarya and neuritic processes of mouse motor neurons, expression of CMT mutant NFL (P8R and Q333P) leads to progressive degeneration and loss of cultured motor neurons. Early fragmentation and loss of neurites in degenerating motor neurons is associated with disruption of the NF network and aggregation of NFL protein. Co-expression of HSPB1 diminishes aggregation and neurotoxicity of CMT mutant NFL and can even reverse aggregation and promote assembly of mutant NFL. Like CMT mutant NFL, expression of CMT mutant HSPB1 (S135F) also leads to progressive degeneration and loss of motor neurons with disruption of NF network and aggregation of NFL protein in degenerating motor neurons. Additional studies show that wild-type and mutant HSPB1 associates with wild-type and mutant NFL and that mutant HSPB1 has a dominant effect on disruption of NF assembly and aggregation of NFL. Finally, and most importantly, we show that deletion of NFL, using primary cultures from NFL null mutant mice, markedly reduces the degeneration and loss of motor neurons due to expression of CMT mutant HSPB1.

Discussion and conclusion: The findings indicate that disruption of assembly and aggregation of NFL is a common triggering event in motor neuron degeneration in CMT2E and CMT2F disease and may be instrumental in promoting selective degeneration of motor neurons in other motor neuron diseases (5).

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C17 TRANSGENIC MOTOR NEURONS DERIVED FROM HUMAN EMBRYONIC STEM CELLS ARE A USEFUL MODEL OF ALS

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Background: Despite extensive studies of transgenic mouse models of ALS, disease mechanisms in ALS are still largely unknown and effective treatments are not available for this fatal neurodegenerative disorder. New human disease models are needed to take advantage of recently developed high-throughput methods that advance basic research and the discovery of new therapies for ALS. The self-renewal capacity and pluripotent nature of human embryonic stem cells (HESC) provides a remarkable potential for understanding and treatment of neurodegenerative disorders. A crucial step in establishing the potential of HESC for ALS is the differentiation into motor neurons. The differentiation into motor neurons has been previously demonstrated only in a few human ESC lines.

Objectives: 1. To show for the first time that the HSF-1 HESC line can be differentiated into motor neurons. 2. To generate a model of familial ALS expressing mutant SOD-1 in HSF-1-derived motor neurons, which will model ALS and can be used to study disease mechanisms and new therapies.

Methods: HSF-1 HESC were initially differentiated into motor neurons with retinoic acid and sonic hedgehog, and further differentiated with brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) and B27. HSF-1 cells were transfected with the lentiviral pLenti6/438Hb9/BGlo/SOD/IRES2/EGFP (pLenti6SOD) vector, containing a ß-globulin/Hb9 promoter /enhancer, which selectively and stably expresses human wild type or mutated G93A, A4V or I113T SOD-1 DNA, and a green fluorescence protein (GFP) reporter in motor neurons. An initial characterization of the new *sod-1* transgenic HSF-1 cell lines included cell death assays and morphological studies to analyze the effect of the mutants on the differentiating and mature motor neurons.

Results: Using immunocytochemistry with neural cell type-specific antibodies, we have shown that most of the differentiated neuronal cells were positive for neural marker βIII tubulin and a subset of cells express motor neuron markers, such as Hb9, Islet1 and Choline acetyletransferase (ChAT). This was also confirmed by RT-PCR analysis. Quantitative cell death and morphological studies show that all SOD-1 mutants result in shorter processes branching from the neural cell bodies and a statistically significant higher cell death rate when the differentiated cells are exposed to serum-starved conditions.

Discussion and conclusions: This is the first report of a successful differentiation of human ESC line HSF-1 into motor neurons. It is also the first report of human stem cell derived transgenic motor neurons that mimic ALS. Further studies to characterize these cells include apoptosis studies and pharmacological screens using small molecule libraries. These cells are invaluable for studies of interactions of glia and motor neurons in ALS.

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SESSION 3B COMMUNICATION AND ASSISTIVE TECHNOLOGIES

C18 ASSISTIVE TECHNOLOGIES FOR SPOKEN AND WRITTEN COMMUNICATION

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The technological revolution has expanded communication options for people with ALS (pALS) who cannot rely on natural speech and writing. The assistive technologies are categorized as Augmentative and Alternative Communication (AAC) devices. A systematic review of AAC devices reveals 4 critical variables that are manipulated within clinical and research domains: language representation, output, motor access, and microprocessor units. *Language representation* has received significant attention for rate enhancement. While most pALS spell and rely on typing as a form of input, they can never approach speech production rates; often the slowness of AAC devices reduces their utility. Devices are being designed now that integrate natural language processing and prediction algorithms for word, utterance and even conversational level units as we try to approach natural speaking rates. *The output mode* has seen advances in speech technology for the storage of digitized voice as well as qualitative improvements to synthetic speech. Voice banking is often considered as an early treatment option, where pALS with intact motor speech skills store their spoken words, phrases, sentences and sounds for future use in customized communication devices. The personalized voice and messages can be used along with standard text-to-speech output to retain the pALS' voice signature. Engineering efforts to customize synthetic speech to the user's own voice through minimal speech sampling is in progress. Speech scientists continue to tackle the gold standard for a device: bad speech in and good speech out, with attention being paid to recognition of dysarthric speech and production of personalized voices (1). *Motor access* problems are being addressed with visual evoked potentials, detection of alpha and theta waves, and eye gaze recognizers so that head, shoulders, knees and toes are no longer needed. Most devices now offer a range of access methods, starting with keyboards, touch screens, a head mouse, and Morse Code, with auxiliary access through a myriad of switches. Finally, *microprocessor units* are available in every shape and size to meet the needs of the user, from PDAs to laptops made of magnesium-alloy shells, to software that can be downloaded from the Internet and accessed through any home computer. Environmental control units, telephones, MP3 music players, and cameras have been built into some devices. Many devices capitalize on the use of text messaging via cell phone technology to allow the user to text message other individuals via cell phone. Internet phone technology, such as Skype, can allow a device to be used as a telephone.

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Clinical outcomes studies address the acceptance and use of AAC technology by pALS and their family members (2,3). Results reveal that messaging for social interaction is as important as directing medical care. Outcomes research indicates that about 75% of pALS use AAC systems: one-third of the users rely on the system for less than 1 year and two-thirds of the sample use AAC for more than 2 years. Clinical pathways are developed to help patients and families decide when to obtain AAC devices during the course of the disease, and how to integrate them into employment, recreation, medical care and personal activities. Within the United States, policies regarding procurement of AAC devices and services have been established so that intervention can be timely and effective.

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C19 ASSISTIVE TECHNOLOGY CLINICAL PROGRAM FOR ALS: TRANSLATING EVIDENCE INTO PRACTICE

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Background: Evaluative studies that assess specialized interventions usually highlight a need for specific programs of care. Evidence from the literature and outcome research studies on assistive technology usage by ALS patients suggest the necessity for the creation of a systematic, population based approach to identify patients, intervene with specific technologies, and measure outcomes. Fundamental to successful implementation is a theoretical base wherein program planning can occur and evaluation is a key component.

Objectives: 1) To create a multidisciplinary, evidence based assistive technology service for ALS patients in partnership with a large ALS clinic in a university teaching hospital. 2) To develop and implement a model that is client centered and takes into consideration the rapid neurodegenerative nature of the disease. 3) To increase

access to assistive technologies through the model implemented. 4) To provide sufficient evidence to generalize and replicate the results by adopting the model in another location.

Methods: 1) Theory of Occupational Performance and CAOT Guidelines for Client Centred Practice in Occupational Therapy (1) were chosen as the theoretical framework. 2) Components of various models were embedded in the theoretical framework to develop the ATC Client Centered Systems Approach. 3) Funds were obtained to implement the model and provide a system whereby the team could intervene from diagnosis to palliation. 4) Impact and summative data was collected and used to estimate the extent of change based on the program interventions. 5) Trends in the data were analyzed within the program and across the province by collaborating with a provincial funding program.

Results: 1) The ATC Client Centered Systems Approach is successful in following ALS patients through the continuum of care and allows the team to intervene when needed. 2) Summative data demonstrated increased prescription of technology. 3) The program has successfully been replicated at another site for patients with other neurological and neurodegenerative conditions.

Discussion and conclusions: Through translation of evidence into practice, the ATC Client Centered Systems Approach was developed. Results have shown that it is an integrated system that is evidence based, follows the client through the continuum of care, and allows teams to proceed temporally from the client's point of entry through to palliation. Based on the evidence, the program has been replicated for other patient populations. Some of the challenges facing the model are maintaining a flexible and responsive system of service delivery while allowing growth. Future research is required with regard to sustainability.

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C20 EFFECTIVENESS OF AND SATISFACTION WITH AN EYE-TRACKING SYSTEM FOR COMMUNICATION IN PATIENTS WITH EXTREMELY SEVERE ALS

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Background: During the course of ALS, the ability to communicate progressively worsens, up to the complete loss of any useful communication.

Objective: To assess the usefulness of and the satisfaction with an eye-tracking system for communication in a series of patients with extremely severe ALS.

Methods: An eye-tracking system for communication was given to a series of ALS patients with a clinically severe disorder. The system included several video keyboards with increasing difficulty. The patients used the system for a week and were assisted by a speech therapist and a psychologist during three two-hours sessions (day 1, day 2, and day 7). At days 1 and 7, the patients were also administered a QoL questionnaire (McGill Quality of life questionnaire, MQoL), a depression rating scale (Zung Depression Scale, ZDS), a scale to evaluate the perception of being a burden (Self-Perceived Burden Scale, SPBS), and a scale evaluating the satisfaction with life (Satisfaction with Life Scale, SWLS). At day 7, they also completed a questionnaire evaluating efficacy, efficiency, satisfaction, and ease of use of the eye-tracking system.

Results: The eye-tracking system was utilized by eighteen patients (mean age, 63.6 years, range 45-81), with a mean ALS-FRS score of 4.2. All patients learned the use of the system quite easily and rapidly. They could use it both for communication and for fun. The major problems were tiredness after one hour of use, and the position they had to maintain with their head. Patients' QoL significantly improved and their level of depression significantly decreased after the use of the eye-tracking system. Patients with some residual movements (finger, hand, foot, head) that could be used for communication with a computer tended to use the eye-tracking system less frequently.

Conclusions: The eye-tracking systems seem to be effective in improving communication for severe ALS patients and in improving their QoL, although they still have technical problems. Eye-tracking systems should be reserved for subjects with no residual possibilities of communication, since patients that could use alternative ways of communication tended to use them less frequently and for shorter periods of time.

C21 DEVELOPMENT OF AN AUTOMATIC RECOGNIZER FOR DYSARTHRIC SPEECH

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Background: Computer access via voice recognition is a notable challenge to people with dysarthria. There are several commercially available automatic speech recognition (ASR) systems for use by typical English speakers, but these systems do not work well with people with speech disorders. This is because the voice recognition models are based on typical English speakers. We are investigating the creation of ASR models based on dysarthric speech.

Objectives: This paper will discuss the research underway to investigate the feasibility of a speaker independent ASR system that is able to recognize imperfect, e.g. dysarthric speech.

Methods: We are collecting samples of dysarthric speech, from which ASR models are created. The models are then evaluated by individuals with dysarthria. Subjects read

digits 0 to 9 which are written out (e.g. EIGHT, FOUR, ZERO) while sitting in front of a computer monitor while wearing a light, head mounted microphone. Subjects read one hundred ''zip codes'' that are simultaneously recorded by a computer. Voice samples are verified by researchers for completeness and accuracy and are then built into ASR models.

Results: To date a total of 50 sample sets have been collected from 29 females and 21 males with dysarthria. This data has been used to create the first set of computer acoustic models based on dysarthric speech samples. ASR models are created from a subset of 80% of each sample set and tested or ''exercised'' with the remaining 20%. Initial correctness numbers are historically where we would expect them to be. Word Recognition Correctness for the models follows: Female Mild =86.24%; Female Moderate =70.27%; Male Moderate =75.38%; Female Severe =51.25%

Discussion and conclusions: While the results from the models are lower than expected, the pattern of decreasing word recognition percentage with increasing dysarthria is expected and demonstrates the models represent the data. The 86% word recognition rate for the Female Mild model and the rates for the Male and Female Moderate models are in line with the initial performance of off-the-shelf ASR systems as reported by Koester, while the Female Severe word recognition of 51% is better than random and shows correlation. Additionally, these models are raw and have not been tuned. We expect that tuning the linguistic model to the acoustic model, as is common practice in ASR systems, will improve the recognition rates. In this study subjects with dysarthria secondary to ALS were chosen specifically because ALS does not generally affect sensory or cognitive abilities. Associated motor impairments did require the development of portable data collection systems enabling researchers to go to the subject, rather than have the subject come to us.

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SESSION 4A SPINAL MUSCULAR ATROPHY

C22 ADVANCES IN SMA RESEARCH

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Spinal muscular atrophies (SMA) are frequent recessive autosomal disorders characterized by degeneration of lower motor neurons and caused by mutations of the *SMN1* gene. *SMN1* is duplicated in a homologous gene called *SMN2* which remains present in patients. Full-length transcripts (SMN^{FL}) are almost exclusively produced by the *SMN1* gene, whereas the predominant form encoded by the *SMN2* gene is lacking exon 7 ($SMN^{\Delta 7}$) leading to a reduced dose of SMN^{FL} in SMA.

SMN facilitates the formation of the spliceosome, a large RNA-protein complex involved in pre-mRNA splicing, transcription and metabolism of ribosomal RNA. In spite of major advances in the biochemistry of SMN, the molecular pathway linking SMN defect to the SMA phenotype remains unclear. Several hypotheses have been tested to explain the vulnerability of motor neurons in SMA with contradictory results.

Recently, we generated new antibodies specific to SMN^{FL} or $SMN^{\Delta 7}$. In transfected cells, we show that the stability of the $SMN^{\Delta 7}$ protein is regulated in a cell dependent manner. Importantly, whatever the human tissues examined, $SMN^{\Delta 7}$ protein was undetectable due to the instability of the protein, thus excluding a dominant effect of $SMN^{\Delta 7}$ in SMA. These data indicate that motor neuron degeneration is caused uniquely by a reduction in SMN^{FL} protein levels.

A similar decreased level of SMN^{FL} was observed in brain and spinal cord samples from human SMA. These data indicate that the vulnerability of motor neurons cannot simply be ascribed to the differential expression, or a more dramatic reduction of SMN^{FL}, in spinal cord when compared to brain tissue. SMN^{FL} may have specific targets in motor neurons.

Upregulation of the *SMN2* gene expression or preventing exon 7 skipping of SMN2 transcripts have been selected as therapeutic strategies in SMA. Our study allowed the identification of a new target consistent in improving the stability of $SMN^{\Delta 7}$ protein in SMA patients. These new polyclonal antibodies specific to the $SMN^{\Delta 7}$ protein should be very useful for the detection of drugs able to stabilize this isoform. Alternatively or in combination with the above strategy, neuroprotective or neurotrophic factors may protect neurons against toxicity or promote axonal sprouting of motor neurons. Several compounds have been identified and should be validated *in vivo* to select the best candidates for therapeutic trials in human SMA.

C23 USING ZEBRAFISH TO ADDRESS THE FUNCTION OF SMN IN SPINAL MUSCULAR ATROPHY

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Background: A critical question in spinal muscular atrophy (SMA) research is why reduced levels of SMN, a ubiquitously expressed protein, leads to a motoneuron-specific disease. It has been hypothesized that SMN may have a dual function: the well-characterized role in mediating snRNP assembly and a novel function in axons. We have previously shown that decreasing Smn levels in zebrafish with antisense morpholinos causes defects in motor axon outgrowth.

Objective: To determine the function of SMN that is critical in SMA

Methods: We use antisense morpholinos and RNA over expression to manipulate specific proteins in zebrafish followed by analysis of motor axons.

Results: Using the zebrafish model, we see a correlation between the severity of the axon defects and survival when Smn levels are decreased. In addition, we also see evidence of synaptic defects in hemisegements with defective motor axons. Using these motor axon defects as an assay, we have shown the mutant forms of SMN that retain snRNP properties fail to rescue these axon defects and forms that do not retain these functions can rescue the motor axon defects caused by low levels of Smn. To determine whether decreasing other components of the snRNP complex would also cause motor axon defects, we knocked down Gemin2, a SMN binding protein involved in snRNP assembly. Moderate knockdown of Gemin2 yields a large percentage of morphologically abnormal embryos with shortened trunks and overall delayed development. Examination of motor axons revealed that only embryos with abnormal body morphology had aberrant motor axons indicating that the motor axon defects are secondary to the overall body defects observed in these embryos. To directly test this, we knocked down Gemin2 specifically in motoneurons using two separate approaches and found that motor axons developed normally. Furthermore, wild-type neurons transplanted into morphologically abnormal *gemin2* morphants had aberrant motor axons indicating that the motor axon defects observed when Gemin2 is decreased are secondary to the defects in body morphology.

Conclusions: These data support the hypothesis that inhibiting snRNP assembly does not lead to defects in

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motor axon outgrowth and are consistent with the hypothesis that Smn function in motor axon development, and perhaps in SMA, is snRNP independent. We will also discuss our ongoing work to generate a genetic model of SMA in zebrafish.

C24 DETERMINING THE CRITICAL FUNCTION OF SMN IN SMA USING MOUSE MODELS

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SMA is caused by loss of the *SMN1* gene and retention of *SMN2* gene. We have replicated this situation in the mouse by placing two copies of *SMN2* onto a mouse Smn null background ($SMN2^{+/+}$; $Smn^{-/-}$). These mice show severe atrophy of muscle and die at 5 days. One copy of *SMN2* results in embryonic lethality whereas 8 copies of *SMN2* rescues lethality and the SMA phenotype. The introduction of *SMN* lacking exon 7 extends survival of SMA mice to 14 days whereas introduction of a mild missense mutation, SMNA2G, extends survival beyond a year resulting in mice with mild SMA. Interestingly, SMNA2G cannot rescue embryonic lethality of *Smn* null animals but can rescue the lethality of single copy *SMN2*, *Smn* null animals indicating that SMN's essential function requires a heteromeric complex. We have crossed SMA carrier mice to mice expressing the GFP:HB9 transgene so as to label motor axons with GFP throughout development. We find that severe SMA mice have completely normal motor neuron outgrowth and axonal pathfinding. This contrasts to what is observed in motor neuron cultures from these mice or in knockdown of SMN in zebrafish. However, we do observe a significant increase in unoccupied synapses of SMA embryos at 18.5 days of development (e18.5) but not at e17.5 indicating denervation occurs prior to birth in these animals. Additionally, overexpression of SMN in neurons of SMA animals rescues the SMA phenotype. However, overexpression of SMN in just muscle fibers has no impact on survival of SMA animals. We are currently using a genetic approach to determine the critical function of SMN in SMA. In addition, we have tested drug compounds in the SMA mouse models we have developed.

C25 A HYPOMOPRHIC PANEL OF SMN MICE THAT RECAPITULATE SMN2 SPLICING TO DEFINE THE DOSAGE AND TIMING REQUIREMENTS OF SMN EXPRESSION

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Background: Spinal Muscular Atrophy (SMA) is a common autosomal recessive motor neuron disorder that is the leading genetic cause of infant mortality. SMA is caused by the loss of the survival motor neuron gene (*SMN1*). *SMN2*, a nearly identical copy gene, is present in all SMA patients but differs by a critical nucleotide that alters exon 7 splicing efficiency. This results in low SMN levels which are not enough to sustain motor neurons. Thus, SMA is not a true loss-of-function disease, but rather one of dosage in which clinical presentation results in severe (Type I), intermediate (Type II) and mild (Type III) forms.

Objectives: The goals of this project are to define SMN dosage requirements for normal health, determine the timing requirements of SMN function and the therapeutic window of treatment for severe and mild forms of SMA.

Methods: We are using mice, which only have one *Smn* gene to titrate SMN dosage and recapitulate the alternative splice of *SMN2* exon 7. To achieve this, we used gene targeting strategies to engineer two different mutations within *Smn* exon 7 splice enhancer elements (ESE). The first allele, $Smn^{C\text{-}T}$, is a C-T nucleotide transition that mimics *SMN2*. The second allele, Smn^{2B}, alters the central ESE within *Smn* exon 7. Both alleles produce a mixture of transcripts, some contain while others lack exon 7 and the amount of splicing depends upon the mutation.

Results: We have used the $Smn^{C\text{-}T}$ and Smn^{2B} alleles in combination with the *Smn* null and wild type alleles to titre Smn dosage from 100-0% in increments as small as 5%. Some mice present with a SMA phenotype and pathology, while others do not. Hence we believe we have identified the minimal amount of Smn required for normal health and maintenance of motor neurons. We will present our current results from these lines. In addition, the progenitor lines of these mice, $Smn^{C\text{-}T/Neo}$ and $Smn^{2B\text{-}Neo}$ contain a LoxP flanked Neomycin resistance cassette in intron 7. Its presence causes exon 7 to be excluded from transcripts; however, these progenitor alleles are ''repairable'' as excision of the floxed Neo cassette by Cre recombinase allows exon 7 to be included into transcripts. Hence these lines of mice are inducible *Smn* alleles that can be used to return Smn expression temporally by combining it with a tamoxifen-inducible Cre line.

Discussion and conclusions: We have generated a titration series of Smn alleles that mimic human SMN2

splicing and these mice have allowed us to determine the dosage requirement of SMN for normal health and how low levels of Smn affect development and maintenance of motor neurons. Importantly, one of our genotypes dies at about one month of age and fills a void in the currently available mouse models. To our knowledge we have also generated the first inducible Smn alleles and importantly they return Smn levels at physiological concentrations, are at the endogenous Smn locus, and mimic human SMN2 splicing. Overall, the reagents and research that we have generated and performed will be very useful for studying the underlying mechanism(s) of SMA and testing of therapies.

C26 DEVELOPMENT AND VALIDATION OF A METHOD FOR THE ABSOLUTE QUANTIFICATION OF SMN1 AND SMN2 FULL LENGTH TRANSCRIPTS: POSSIBLE APPLICATION FOR CLINICAL TRIALS FOR SPINAL MUSCULAR ATROPHY

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Background: To date it is not definitely established whether SMN quantification, at transcript or protein levels, can be considered a reliable biomarker to monitor the response to treatment of spinal muscular atrophy (SMA) patients. Although several tests, mainly based on real time PCR, have been developed, none of these allow demonstration of whether a clear-cut difference exists in SMN full length (SMN-fl) transcript levels among controls, carriers, and patients.

Objectives: To develop and validate a new molecular test based on absolute *SMN1*-fl and *SMN2*-fl transcript quantification by real-time PCR.

Methods: Our method exploits techniques which have been used for several years to determine the plasmatic load of some RNA viruses. *SMN1*-fl and *SMN2*-fl transcripts have been quantified as number of molecules/ng of RNA; *GAPDH* quantification was included to evaluate both RNA sample quality and RT-PCR efficiency.

Results: At present, we have determined *SMN1*-fl and *SMN2*–fl transcript levels in 23 healthy controls, 21 carriers and 24 patients. In controls (11 females and 12 males), average *SMN1*-fl and *SMN2*-fl levels were 78.72 ($\pm$53.90), and 38.29 ($\pm$24.69) mRNA molecules/ng of RNA, respectively. In carriers (14 females and 8 males), we observed a mean of 72.00 ($\pm$37.56), and 58.70 ($\pm$43.30) mRNA molecules/ng of RNA for *SMN1* and *SMN2*, respectively. The differences between carriers and controls were not significant ($p=0.63$ for *SMN1* and $p=0.06$ for *SMN2*). We have also analyzed blood samples from 9 SMA type II and 15 type III patients. SMA type III patients showed on average higher levels of *SMN2*-fl transcripts compared to SMA type II (64.82 ± 30.47 vs. 42.86 ± 12.43 mRNA molecules/ng of RNA, respectively; $p=0.053$). When considering all patients as a group, the average number of *SMN2*-fl molecules was 56.58 ($\pm$27.15)/ng of RNA, higher than that found in controls ($p=0.02$). This finding may be hypothetically related either to an increase in *SMN2* expression in leukocytes, to compensate the absence of *SMN1*, or to differences in *SMN2* copy number. The most relevant finding of this study is that both SMA type II and type III patients showed a significant reduction of *SMN2*-fl compared to total SMN-fl (*SMN1*-fl plus *SMN2*-fl) transcript levels of controls and carriers ($p=0.008$ and $p=0.018$, respectively). The analysis of further patients, including SMA type I, as well as *SMN2* gene copy number assessment is currently ongoing.

Conclusions: We have developed a highly sensitive assay for the determination of *SMN1* and *SMN2* full length transcripts in blood. We have demonstrated for the first time, a significant reduction of SMN-fl transcript levels in blood leukocytes of type II/III patients with respect to control individuals, suggesting that SMN level determination could be a suitable biomarker for the evaluation of response of patients to pharmacological treatment in SMA patients.

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SESSION 4B COGNITIVE AND PSYCHOLOGICAL CHANGE

C27 ANXIETY, DEPRESSION AND QUALITY OF LIFE DURING THE DIAGNOSTIC PHASE OF ALS PATIENTS AND CAREGIVERS

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Background: There are few studies about the psychological status of ALS patients and their caregivers during the diagnostic phase, which is likely to be a period of great worry and distress.

Objective: To evaluate anxiety, depression and quality of life (QoL) during the diagnostic and follow-up phases in ALS patients and their caregivers.

Methods: A total of 75 ALS patients and their primary caregivers were interviewed separately. State and Trait Anxiety Inventory (range 20–80), Zung Depression Scale (range 20–80), McGill Quality of Life Questionnaire (MQOL) (range 1–10), Satisfaction With Life Scale (SWLS) (range 5–35) and Idler religiousness/spirituality scale (range 4–17) were assessed. Patients' physical status was evaluated with ALS-FRS (range 0–40). Student's t-test and linear regression analyses were performed (SPSS 12.0; $p < 0.05$).

Results: Forty-six patients were men and 29 women;.29 were interviewed in the diagnostic phase and 46 during the follow-up; patients mean age was 63.6 [9.2], their mean ALS-FRS was 29.7 [7.2]. Patients' mean scores were: ZDS, 42.5 [8.3] with 18% of subject above the fifty-point cut-off score indicating overt depression; MQOL, 6.8 [1.3]; SWLS, 28.0 [5.9], state anxiety, 45.2 [12.4] (Italian population norm, 41.7); trait anxiety, 41.2 [10.1] (Italian population norm, 41.5); Idler, 10.4 [3.3]. A significant difference between state anxiety during the diagnostic phase and state anxiety during the follow up was found in patients ($p < 0.01$). Caregivers Satisfaction with Life score was higher in the diagnostic phase then during the follow up ($p < 0.05$). In multivariate analysis, factors related to patients' QOL were depression ($p < 0.01$), state anxiety ($p < 0.01$), religiousness ($p < 0.01$), caregivers' QOL ($p < 0.05$) and caregivers' physical well-being ($p < 0.05$).

Conclusions: State anxiety is higher in ALS patients during the diagnostic phase, and independently influences their QOL. Our findings provide evidences of the influence of caregivers well-being and QOL on patients' QOL. Therefore, it is important to assess and aggressively treat anxiety in ALS patients, especially during the early phases of the disorders.

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C28 FACTORS RELATED TO HEALTH LOCUS OF CONTROL IN ALS PATIENTS: A CROSS-SECTIONAL STUDY

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Background: Health locus of control (HLC) represents a person's belief regarding control over his/her illness. It measures the extent to which an individual believes that external factors and internal factors play important roles in determining his/her health.

Aims: To perform a cross-sectional evaluation of HLC in a series of ALS patients, and to assess its determinants.

Methods: ALS Patients underwent a battery of questionnaires. Locus of control was evaluated with the Multidimensional Health Locus of Control (MHLC) scale, Form C. The MHLC includes 3 domains (internal [MHLC-I], chance [MHLC-C], powerful others [MHLC-PO], the latter being subdivided in two subdomains, doctors [MHLC-D], and other people [MHLC-O]). Depression and anxiety were evaluated with the Hospital Anxiety and Depression Scale (HADS) and the Zung Depression Scale (ZDS). The cognitive and emotional representation of illness was evaluated with the Brief Illness Perception Questionnaire (Brief IPQ). Quality of life was assessed with the McGill Quality of Life Questionnaire (MQoL). Gender, age, years of formal education, disease duration, and ALS-FRS score were also evaluated.

Results: A total of 54 consecutive ALS patients were evaluated (29 men and 25 women, mean age 62.0, SD 12.2). The mean MHLC-I score (16.5; SD 4.6) was significantly lower than the mean MHLC-C score (24.3; SD 6.7) ($p=0.0001$), and both were lower than the MHLC-PO score (29.3; SD 5.2) (both $p=0.0001$). In multivariate analysis, MHLC-I score was related to a higher number of formal education years ($p=0.004$) and a higher ALS-FRS score ($p=0.012$); MHLC-C was related to older age ($p=0.004$); female gender ($p=0.008$), and high scores in the treatment and timeline domains ($p=0.01$ and $p=0.033$ respectively); MHLC-PO was related to a higher total MQoL score ($p=0.013$), and a older age ($p=0.049$). In the Brief IPQ, the mean higher score was obtained for the treatment control domain and the lower for the timeline domain, indicating that the patients have a clear perception of the progressive nature of their disorder and that they tend to trust in the possibility of therapeutic interventions.

Conclusions: Our ALS patients tend to have an external HLC, with a particularly high level relative to the role of

powerful others. An internal HLC has been found in patients with a higher educational level and a higher ALS-FRS score (i.e., subjects with a better clinical status). Both internal and external HLC were not influenced by depression, anxiety, or disease duration.

C29 COMPARISON OF PATIENT AND FAMILY BEHAVIOURAL REPORTS IN ALS PATIENTS WITH AND WITHOUT FTD

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Background: Behavioural abnormalities are a common feature of FTD but have only rarely been studied in ALS. Poor insight is one of the five core symptoms of the Neary criteria for FTD but systematic examination of insight has not yet been studied in the ALS population and, to date, caregiver reports have generally been utilized in lieu of patient reports because of concern that patients with frontal dysfunction may lack insight.

Objectives: To compare the type and degree of behavioural changes endorsed by patients and family members, and to compare differences between ALS patients with and without FTD using the Frontal Systems Behavior Scale (FrSBe).

Methods: Ten patients with ALS who were referred for comprehensive neuropsychological assessment completed the Self Rating Form of the FrSBe, while a family member completed the Family Rating Form. The FrSBe provides ratings for both premorbid and current behaviour across three domains of Apathy, Disinhibition and Executive Functioning, as well as a Total score. Raw scores for the Self and Family rating forms were transformed into standardized T scores. Analyses were completed on three ALS patients diagnosed with probable FTD by Neary criteria and on seven patients with ALS who did not have FTD.

Results: No significant differences existed between patient and family behavioural ratings on any of the FrSBe subscales or total scores in ALS patients without FTD. Both patients and family members endorsed borderline significant elevations on Apathy, Executive Dysfunction and Total scores. The three ALS patients diagnosed with probable FTD also provided ratings consistent with their family members regarding premorbid behaviour. However, family members endorsed extreme behavioural abnormalities on Apathy, Executive Dysfunction and Total subscales since the onset of ALS compared to self ratings by patients. On average, family members reported 3.6 standard deviation (sd) greater behavioural dysfunction than the FTD patients on the Apathy scale, and more than 2 sd greater behaviour dysfunction on the Executive Dysfunction subscale and the Total score.

Discussion and conclusions: Among ALS patients who do not have FTD, there is general concordance between patients and family members regarding behavioural change using the FrSBe. Patients and caregivers reports show that apathy and executive function changes are common, but the changes are mild. In contrast, reports by patients with probable FTD show significantly lower levels of behavioural dysfunction than reports by family members, suggesting a lack of insight in this cohort, while family members reported levels of Apathy, Executive Dysfunction and Total scores that were significantly higher than ALS patients who did not have FTD. The findings suggest that by demonstrating behavioral changes and lack of insight, the FrSBe can be useful for the diagnosis of FTD in patients with ALS.

C30 NEUROPSYCHOLOGICAL FUNCTIONING IN PRIMARY LATERAL SCLEROSIS: A COMPARISON WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Primary lateral sclerosis (PLS) is a rare adult-onset motor neuron disease. Although the contemporary view of amyotrophic lateral sclerosis (ALS) is that of a multisystems disorder in which cognitive impairment is increasingly observed, the evidence for cognitive impairment in PLS is less well established.

Objectives: In order to characterize the extent of neuropsychological dysfunction in PLS, we studied prospectively cognitive, emotional, and behavioural functioning in PLS, and compared performances to functioning in ALS.

Methods: Eighteen patients with PLS and 13 patients with ALS completed a neuropsychological battery assessing both cognitive skills and emotional/behavioural functioning. The test battery was categorized into 5 general domains with particular emphasis on frontal-executive skills but also including assessment of attention/concentration; visual-perception/constructional skill; memory; and emotional/behavioural functioning. Tests/procedures were chosen to minimize speech production and motor requirements.

Results: All scores were standardized into T-scores based on normative data corrected for age and education when

possible. A T-score less than 35 (i.e., >1.5 SD below the mean) on a specific measure was considered abnormal. A patient's overall performance on the test battery was defined as cognitively impaired if 2 or more scores were abnormal on at least 2 different cognitive tests. Although both the PLS and ALS groups scored broadly within normal limits on all cognitive measures, there was considerable heterogeneity amongst both groups when data were examined on a case by case basis. Overall, 39 percent of PLS patients and 31 percent of ALS patients were considered cognitively impaired. The most affected areas of cognition were highly similar in both groups, and included oral word fluency (the most sensitive measure), facial recognition memory, and verbal source memory. A majority of PLS patients also exhibited abnormal behavioural symptoms, and the groups were also similar with respect to emotional and behavioural symptoms. There was no relationship in either group between cognitive functioning and disease duration, current site of disease, site of onset, functional status, and respiratory variables.

Discussion and conclusions: Although not all PLS patients demonstrate cognitive dysfunction, cognitive impairment appears to be a common symptom of the disease as with sporadic ALS. More than one third of patients with PLS were cognitively impaired and more than half had abnormal behavioural symptoms. Although not in the range of frontotemporal dementia, deficits were most prominent on tests of executive functioning. Direct comparison between patients with PLS and ALS suggests that the frequency and pattern of cognitive dysfunction and behavioural changes is remarkably similar in both diseases. We believe the impairment reflects a dysexecutive syndrome resulting from dysfunction of frontal lobe circuitry. The findings of this study lend support to the concept that PLS and ALS belong to a continuum of disease rather than separate disease processes.

C31 A NEW MEASURE OF PSEUDOBULBAR AFFECT REVEALS HIGH PREVALENCE RATES IN A SAMPLE OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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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 CVA, multiple sclerosis and Amyotrophic Lateral Sclerosis (ALS) patients. The Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report measure of PBA, is a commonly used instrument to measure PBA, but it is limited in scope and depth.

Objective: To design a more comprehensive PBA questionnaire (PBAQ) with both patient and caregiver-rated components, to identify a wider range of clinical symptoms in PBA, test for correlations with ALS disease features, and to compare the PBAQ with the previously validated CNS-LS.

Methods: Patients were recruited consecutively from a multidisciplinary ALS Center, and requested to complete the CNS-LS and PBAQ, as part of a larger screening study for cognitive and behavioral changes in ALS patients.

Results: Using a similar clinical cut-off as the CNS-LS, requiring weekly episodes of labile emotion, patients and/or caregivers in this sample responded on the PBAQ that 52% of the sample had clinical levels of PBA (15/29). In contrast, 34% of the sample (n=10) had clinical levels of PBA as measured by CNS-LS scores of 13 or above. Of those patients and caregivers who rated PBA as a weekly problem on the PBAQ, 47% (7/15) also rated uncontrollable outbursts of anger and irritability as another problem. Patients reported more crying than laughing episodes, they could "almost always" identify a specific trigger for episodes, and they had "somewhat" control over episodes once they began. There were no statistical associations between PBA and level of depression, sex, education level, age, length of illness, onset site, illness severity, or breathing capacity. There was also no statistical association between PBA and cognitive functioning, as measured by a word generation measure.

Discussion and conclusions: PBA is a disabling syndrome for many ALS patients yet is well treated with available medications. The PBAQ may be a more sensitive measure that identifies more patients struggling with this problem, by questioning both the patient and caregiver. As nearly half of the families coping with PBA also endorsed uncontrolled anger/irritability, the concept of PBA may be expanded to include a third feature of emotional lability: anger. The PBA-Q may allow clinicians to better identify patients with PBA who lack insight into their behavior and better characterize the nature of the episodes, thus improving clinical management.

C32 THE CAMBRIDGE BEHAVIOURAL INVENTORY IN EVALUATING FRONTOTEMPORAL FUNCTION IN PATIENTS WITH MOTOR NEURON DISEASE

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Background: Frontotemporal dysfunction (FTDys) may occur in patients with motor neuron disease (MND), though the nature of the symptoms together with the patients' physical limitations from their MND may make it difficult to detect. Caregiver questionnaires, including the Cambridge Behavioural Inventory (CBI), have been used to help detect the behavioural and neuropsychiatric manifestations of FTDys (1).

Objective: To describe the patterns of involvement in the domains assessed by the CBI in our patients with MND.

Methods: We retrospectively analyzed CBI questionnaires filled out by caregivers of 26 of our patients with MND at the time of their first visit to our clinic.

Results: Nine (35%) of the patients were women and 17 (65%) were men with a mean age of 59 ± 12 years. There are 13 domains in the CBI but several are potentially invalid in the MND population, namely everyday skills, self care and sleep. With these excluded, mood was the most common domain involved, with 22/26 (85%) patients having altered mood; the most common alterations were sad or depressed (21/26), restless or agitated (14/26), and irritable (12/26). Poor motivation was reported in 16/26 (62%), and the questions reported abnormal most often were less enthusiasm for interests (13/26), and little interest in doing new things (13/26). Stereotypic and motor abnormalities were noted in 15/26 (58%) patients, including 11/26 with rigid ideas and 8/26 with fixed routine behaviours. Poor memory was reported in 11/26 (42%) with the most common abnormalities being 9/26 losing things and 6/26 repeatedly asking the same questions. Changes in orientation and attention were described in 11/26 (42%), including 6/26 having problems staying on task and 6/26 becoming confused in familiar surroundings. Ten of 26 (38%) were described as having altered eating habits, challenging behaviour in 9/26 (35%), altered beliefs in 6 (23%), disinhibition in 4 (15%), and altered insight and awareness into either memory or behaviour changes in 4 (15%). After the first 20 forms were filled out, we added questions about altered insight into weakness, speaking, breathing, or swallowing, and 2/6 (33%) reported some loss of awareness to problems in at least one of these areas.

Conclusions/Relevance: Patients with MND may show changes in various cognitive domains as measured by the CBI, most often involving mood, motivation, and stereotypic and motor abnormalities. Adding questions pertaining to insight into MND related problems may be useful in detecting altered awareness. Comparing patterns of change in the CBI in patients with MND and those with only FTDys may be of interest in future studies.

Acknowledgements: Supported by the J Thomas May ALS Research Fund.

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SESSION 6A NERVE-MUSCLE INTERACTION

C33 CONNECTOMIC TOOLS FOR *IN SITU* STUDY OF ALS/MND

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The axons that connect motor neurons and their muscle fiber targets are easily accessible in animals, nonetheless the precise pathways and branching patterns of individual motor axons is poorly understood. My laboratory has a longstanding interest in the development of these branching patterns as they undergo a substantial reorganization in early postnatal life. More recently we have begun to analyze these branches in both aging and a mouse model of ALS. Our interest here is to better understand the ways in which disease and aging are compensated by changes in the remaining motor neurons. To study these branching patterns we have developed transgenic animals in which individual neurons express random combinations of several different spectral variants of fluorescent proteins. These *Brainbow* mice allow each motor neuron's axon to be tracked long distances as it expresses a unique color. To track axons long distances it has also been necessary to develop semi-automated image processing tools to identify the same axon in multiple images. Our image data for a single muscle and its nerve may consist of ~100 Gb of image data and tens of thousands of confocal images from hundreds of image stacks. The reconstructions show many surprises about the ways axons are organized and branch on their way to, and within muscles. Recently we have also begun to develop automated methods for serial electron microscopy based on a new microtome we have designed. My colleagues and I are hopeful that these imaging tools will provide insights into the ways in which the peripheral nervous system accommodates neuronal loss.

C34 SELECTIVE DENERVATION OF THE LARGEST MUSCLE FIBERS WITH EARLY LOSS OF THE MOST FORCEFUL MOTOR UNITS IN A MOUSE MODEL OF ALS

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Background: Anatomical studies indicate that the largest caliber motor axons are most vulnerable to die-back in both human patients and transgenic mouse models of Amyotrophic Lateral Sclerosis (ALS). In contrast, electromyographic studies of functional motor units have failed to uncover selective loss and disagree regarding the time-course of die-back.

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Objective & Method: In order to evaluate whether there is selective loss of some but not other motor units during the presymptomatic phase of the disease, prior to onset of symptoms at 90 days of age, we have combined electrophysiological and immunohistochemical methods to enumerate and characterize functional motor units in fast-twitch tibialis anterior (TA), medial gastrocnemius (MG), and the extensor digitorum longus (EDL) muscles and the slow-twitch Soleus (SOL) muscle in the $SOD1^{G93A}$ mouse model of familial ALS.

Results: We found that there was a dramatic decline in the number of motor units in the fast-twitch but not the slow-twitch muscles from 40 days onward to plateau during the symptomatic phase of the disease. Motor units were lost only during the symptomatic phase of disease in the SOL muscle. These data provided strong evidence for a preferential loss of the fast motor units in fALS during the asymptomatic phase of disease prior to 90 days. A parallel decline in the isometric contractile force of the fast-twitch muscles indicated that axonal sprouting was ineffective in reinnervating denervated muscles to compensate for the loss of the functionally intact motor units. At 60 days of age, 30 days prior to the reported onset of symptoms, the number of motor units in $SOD1^{G93A}$ mouse TA muscle was reduced by ~60%. At this age, we found that the most forceful motor units were lost preferentially such that the mean force of the intact motor units decreased to ~50% of the control $SOD1^{wt}$ control mice. Calculation of the mean innervation ratio (IR), the number of muscle fibers innervated per motor unit indicated a trend to increase, consistent with the reported sustained capacity for sprouting of the motor nerves to the type I and type IIA muscle fibers but the inability for nerves to type IID/X and IIB muscle fibers to sprout (1). The reduction in mean motor unit force was accounted for by the preferential denervation of the largest type IIB muscle fibers and an activity-dependent conversion of a proportion of the remaining type IIB muscle fibers to the smaller type IIA muscle fiber type. Analysis of axon sprouting in the muscles, using silver choride/acetylcholinesterase staining, demonstrated that axonal sprouting reinnervated only ~5% of the denervated endplates, thereby accounting for the decline in whole muscle force and the shift of the motor unit force frequency distributions to smaller forces.

Conclusion: Our findings demonstrate that a) the most rapid loss of motor units occurs during the asymptomatic phase of disease, b) the less forceful motor units are preferentially spared in pre-symptomatic $SOD1^{G93A}$ and c) the surviving motor units do not undergo compensatory functional enlargement.

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C35 IDENTIFICATION AND CHARACTERISATION OF A CANDIDATE GENE FOR OSTES, A NOVEL MOUSE MUTANT SHOWING MUSCLE DENERVATION AND ATROPHY

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Background: To facilitate the identification of novel genes involved in MND and neuromuscular disorders, we have exploited *N-ethyl N-nitrosourea* (ENU) mutagenesis to first identify new mouse models of motor neuron loss and muscular atrophy. The *ostes* mouse mutant was generated from this programme. *Ostes/ostes* mice are small, show tremors, and display a unique and complex phenotype of denervation and abnormal polyinnervation of motor endplates and muscular atrophy (see P178 of this supplement).

Objectives: The main objective of this research is the identification of the genetic defect in *ostes* mice. This would further understanding of the genetic and physiological causes of MND and neuromuscular disorders and could suggest new genetic pathways for therapeutic intervention.

Methods: Genetic mapping and haplotype analysis of affected and unaffected mice was carried out using polymorphic genetic markers. Mutation screening was carried out by sequencing and by dHPLC-analysis of DNA from *ostes/ostes* mice. Expression analysis was carried out using real-time PCR. Protein analysis was carried out by Western blot analysis. Transgenic mice carrying a bacterial artificial chromosome (BAC) were generated by oocyte microinjection.

Results: We genetically mapped the *ostes* mutation to a 0.8Mb region on distal mouse chromosome 8. Intriguingly, no mutation was revealed upon coding sequence analysis of all 9 genes included in the region, suggesting that a regulatory mutation may underlie the *ostes* phenotype. In addition, expression analysis by real-time PCR of wildtype and *ostes/ostes* muscle did not show any difference in any of the genes. However, within this genomic segment we identified Pkd1l2 as a candidate gene and generated antibodies against the corresponding protein. Western blot analysis revealed a 200kDa band which matched the predicted size of a known Pkd1l2 isoform that was present in muscle from wildtype mice, but reduced in muscle from *ostes/ostes* mice, and over-expressed in transgenic mice carrying a BAC that contained the full-length Pkd1l2. Subsequent genetic crosses involving BAC transgenics and *ostes/ostes* mice showed that a) the BAC rescued the *ostes/ostes* phenotype and b) homozygous BAC transgenics displayed severe muscle wasting, periodic paralysis and juvenile lethality.

Conclusion: Our results indicate that misregulation of the Pkd1l2 protein underlies the *ostes* phenotype. Further work will address the mechanism of Pkd1l2 dysfunction and how this causes denervation and abnormal innervation with muscle degeneration. No other neurodegenerative or neuromuscular mouse mutants or human disorders map to the *ostes* mouse interval or human region of synteny, respectively. We suggest that Pkd1l2 is essential for the regulation of muscle innervation and function and is therefore a candidate gene for uncharacterised forms of motor neuron disease.

C36 AXONAL DEGENERATION AND OXIDATIVE STRESS: REVISITING THE SOD1 KNOCKOUT MOUSE

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Background: Distal motor axons of SOD1 mutant mice degenerate early in disease, prior to symptom onset, and degeneration proceeds in a distal to proximal, or ''dying-back'' pattern. The mechanism of mutant SOD1-mediated axonal degeneration, and the relationship between axonal degeneration and neuronal cell death in ALS, remains unclear. Previous studies suggest that SOD1 knockout (KO) mice, which do not develop full-blown paralysis, do exhibit pathologic and physiologic signs of a distal motor axonopathy (1–3). This suggests that SOD1 is important for the maintenance of the distal motor axon, presumably through its role as an antioxidant, and raises the question of whether oxidative stress may also cause axonal degeneration in SOD1 mutant mice.

Objectives: 1) To quantify denervation at the neuromuscular junction (NMJ) of SOD1 KO mice, and 2) to evaluate the contribution of oxidative stress to axonal degeneration in primary neuronal cultures from SOD1 KO mice.

Methods: NMJ innervation was quantified in gastrocnemius and tibialis anterior muscles of SOD1 KO mice from 1 month to 18 months of age, compared to SOD1 heterozygote littermates and age-matched wild-type animals. Sciatic nerve, ventral and dorsal roots, and lumbar spinal cord were also analyzed at each time point to monitor for proximal pathology. Primary dorsal root ganglion (DRG) cultures were established from SOD1 KO and heterozygote littermates at postnatal day 4, and axonal outgrowth and survival measured over time at baseline, and in the presence of antioxidants.

Results: By 4 months of age, approximately 50% of SOD1 KO endplates were denervated, progressing to 65% denervation at 1 year, and 90% denervation at 18 months, at which time animals demonstrated significant distal hindlimb weakness. Degeneration of proximal axons was not seen at any time point. There was gliosis and ubiquitinated inclusions in the lumbar spinal cord at 18 months, but not at earlier times. In primary culture, SOD1 KO DRGs extended axons over the first 24–48 hours, and

then degenerated rapidly compared to axons from heterozygote littermates.

Discussion and conclusions: Lack of SOD1 causes accelerated, age-dependent denervation at the NMJ, on a faster time scale than the denervation seen in aging wild-type mice. Reactive pathological changes in the spinal cord of SOD1 KO mice were seen at 18 months. SOD1-KO axons also degenerated rapidly in culture. Taken together, these results suggest, both *in vivo* and *in vitro,* that SOD1 is essential for the maintenance and health of peripheral nerve axons. This raises the question of whether similar mechanisms of axonal death may be involved in mutant SOD1 mouse models of ALS.

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SESSION 6B EVALUATING UNPROVEN TREATMENTS

C37 EVALUATING THE RISKS AND BENEFITS OF UNPROVEN TREATMENT

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Background: In the United States, ''Complementary and Alternative Medicine (CAM)'' is defined by the federal government as ''a group of diverse medical and health-care systems, practices, and products that are not presently considered to be part of conventional medicine''(1). The use of CAM has increased in recent years, with one in three Americans reporting that they use at least one form of CAM (2).
In our clinical experience, we have also observed that many individuals with ALS/MND self-prescribe alternative treatments, especially dietary supplements. As health care providers, we have the responsibility to provide our patients with sound information to make informed decisions about treatment modalities and products.

Objectives: 1) To define the CAM modalities most commonly used by patients with motor neuron diseases. 2) To describe existing evidence concerning the safety and efficacy of those modalities. 3) To provide practical resources for practitioners to evaluate CAM claims.

Methods: Patients presenting to the ALS Clinic for quarterly follow-up visits between May 2007 and July 2007 will complete anonymous written surveys to elicit their utilization and anecdotal experience with therapies identified as CAM in the United States.
A systematic review of the literature will be conducted for scientific evidence to support or refute the most commonly used treatments.

Results: Survey data from our patient population will be compared to national survey data from civilian non-institutionalized American adults.

Discussion: The CAM modalities most commonly utilized by our patients will be described at the Symposium. A review of scientific studies relating to these treatments will be presented along with guidelines and teaching tools for practitioners to educate patients about CAM therapies that may be helpful or harmful. Honest, informed discussion with our patients will help them become wise consumers of CAM and will help us to foster an integrative approach that gleans the best aspects of both CAM and conventional medicine.

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C38 UNPROVEN CELL-BASED TREATMENTS FOR ALS/MND: LESSONS FROM BEIJING

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SESSION 7A AXON MAINTENANCE

C39 NEW PERSPECTIVES ON AXONAL MAINTENANCE IN MOTOR NEURONS

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In the last 5 years the prominence of early axoterminal pathology in motor neuron diseases has become increasingly clear. This presentation will review the current understanding of how axons are normally maintained. Lessons from genetic diseases in man and from genetically engineered animals include the following:

1) Defective axonal transport, especially retrograde transport, can lead to initial axoterminal degeneration and later loss of the neuron. Abnormalities in p150, dynamitin, dynactin, and other molecules of the retrograde motors can produce motor or motor/sensory axonal degeneration. Although the biological effects of these molecules are broad enough that other effects could underlie neuronal diseases seen with mutations on these genes, defective retrograde transport is an attractive candidate mechanism.
2) Defective growth factor signalling can led to distal axonal degeneration. Long a controversial issue, it is now clear that block of Trk signalling can lead to distal axonal degeneration in at least some neuronal populations. In addition, blockade of growth factor signaling prevents collateral sprouting and impairs regeneration after axotomy. Failure of delivery of the growth factor/receptor signaling endosomes to the axon and cell body may be one of the mechanisms by which defective retrograde transport injures axons and impairs regeneration.
3) Mitochondrial abnormalities can lead to early distal axonal degeneration. The responsible defects include abnormalities in mitochondrial fission and fusion.
4) Myelination is axonoprotective, and demyelination produces vulnerability to axonal degeneration. The myelin-associated glycoprotein MAG is one glial molecule involved in axonal protection.

Understanding the basis for axoterminal degeneration in ALS has implications for prolonging motor function and survival.

C40 PROTEOMIC ANALYSIS OF SCHWANN CELLS EXPRESSING MUTANT SOD1

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Background: A potential interplay of spinal motor neurons and non-neuronal cells is proposed in SOD1-mediated ALS pathogenesis, however the role of myelinating glia has received limited attention to date. Schwann cells are principally involved in peripheral nerve myelination, but also govern axonal transport, neurotrophic support and neuromuscular junction stability – salient features involved in early pathology in transgenic mutant SOD1 mice. Importantly, Schwann cells are intrinsic to the co-ordination of motor neuron regeneration after lesion or disease. The pathogenic potential of Schwann cells is already realised by the Charcot-Marie-Tooth type 1 disorders caused by mutations in non-neuronal genes. Thus, myelinating and non-conductive functions of Schwann cells may be targets for mutant SOD1-induced toxicity in ALS.

Objectives: To investigate the impact of wild-type or mutant SOD1 overexpression on Schwann cells using a proteomic strategy and correlate these outcomes to the PNS of transgenic ALS mice.

Methods: Primary Schwann cells were cultured from neonatal rats and high purity was confirmed by S100 immunolabelling. Cultures were transfected with human SOD1 wild-type or G93A vectors. Protein extracts were subject to 2D fluorescence difference gel electrophoresis (DIGE) and differentially regulated spots were analysed by Decyder software and MALDI TOF/TOF mass spectrometry. Protein expression changes were validated by Western blotting and immunocytochemistry of primary cultures and sciatic nerves from constitutive or recently generated Schwann cell-conditional SOD1 expressing transgenic mice.

Results: Proteomic analysis revealed alterations unique to mutant SOD1 expression in Schwann cells, including upregulation of chemokines, neurotrophins and apoptotic factors. These findings, namely p75 neurotrophin receptor signalling, c-jun N-terminal kinase phosphorylation and caspase-3 activation, were substantiated in sciatic nerves of transgenic SOD1 G93A mice at presymptomatic, onset and endstage phases of disease.

Conclusions: These data suggest that mutant SOD1 expression affects the Schwann cell proteome by misregulating neurotrophic and cell death pathways, suggesting neurodegenerative potential of these peripheral glia in ALS. The consequences of Schwann cell protein changes on axons will be addressed by mouse functional studies.

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C41 DYSFUNCTION OF DYNEIN/DYNACTIN PROTEIN COMPLEX AND MOTOR NEURON DISEASE PHENOTYPES CAUSED BY THE DYNACTIN P150^{GLUED} G59S MUTATION IN A NOVEL MOUSE MODEL

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Background: The G59S missense mutation at the conserved microtubule-binding domain of p150^{glued}, a major component of dynein/dynactin complex, has been linked to an autosomal dominant form of motor neuron diseases (MND).

Objective & Method: To study how this mutation affects the function of the dynein/dynactin complex and contributes to motor neuron degeneration, we generated p150^{glued} G59S knock-in mice.

Results: We found that the G59S mutation destabilizes p150^{glued} and disrupts the dynein/dynactin complex, resulting in early embryonic lethality of homozygous knock-in mice. Heterozygous knock-in mice, which develop normally, display MND-like phenotypes after 10 months of age, including excessive accumulation of cytoskeletal and synaptic vesicle proteins at neuromuscular junctions, loss of spinal motor neurons, increase of reactive astrogliosis, and shortening of gait compared with wild-type littermates and age-matched p150^{glued} heterozygous knockout mice.

Conclusions: Our findings indicate that the G59S mutation in p150^{glued} specifically abrogates p150^{glued} activity and accelerates motor neuron degeneration.

C42 MUTANT SPASTIN REDUCES AXONAL TRANSPORT OF MITOCHONDRIA

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Background: Considerable evidence exists for the involvement of defective axonal transport in the pathogenesis of several motor neuron disorders, including Hereditary Spastic Paraplegia (HSP) and Amyotrophic Lateral Sclerosis (ALS). HSP comprises a group of neurodegenerative diseases leading to progressive spasticity and weakness of the lower limbs. Mutations in many different genes have been identified in HSP families, however mutations in the spastin gene are the most prevalent, accounting for approximately 40% of autosomal dominant cases. There is growing evidence for a microtubule-severing function of spastin. Several different models of mutant spastin in cells, *Drosophila* and zebrafish have shown the expression of disease-causing mutants and/or the knockdown of spastin to result in axonal defects. These data suggest a loss of spastin microtubule-severing function, and an increase in the stability of microtubules, is responsible for this deficiency. We therefore hypothesised that mutant spastin-mediated alterations in microtubule stability would result in alterations in axonal transport.

Objectives: To gain insight into how mutations in spastin lead to axonal degeneration in HSP by quantification of axonal transport of mitochondria in neurons in the presence or absence of mutant spastin.

Methods: Primary cortical neurons were prepared from E17 Wistar rat embryos. Neurons were co-transfected with DsRed2-Mito and either EGFP-spastin wild type, EGFP-spastin K388R, EGFP-spastin R424G, or EGFP empty vector, using calcium phosphate precipitation. Live imaging was conducted 18hrs post-transfection and individual cells were recorded for 10 min with 3 s time-lapse interval. The overall distribution of mitochondria along the axon, as well as various properties of mitochondrial motility, including velocity, frequency and duration of movement, were analysed in transfected neurons.

Results: Mitochondrial density per micron of axon was significantly higher in the presence of K388R and R424G mutants. Mutant spastin significantly reduces the velocity of mitochondrial movements in both anterograde and retrograde directions. The frequency of mitochondrial movements are significantly decreased, accompanied by a significant increase in the number of stationary mitochondria. In addition, the time mitochondria spend pausing between movements is significantly longer in the presence of mutant spastin.

Discussion: Together these data are the first to show that mutation of the spastin gene shifts the normal equilibrium between motile and stationary mitochondria. This process therefore alters the dynamics of axonal transport and is likely to play a role in the pathobiology of HSP. The results described here demonstrate that defective axonal transport is a common feature of motor neuron disorders, and provide insight into the pathogenesis of HSP.

C43 REAL TIME IN VIVO ANALYSIS OF RETROGRADE TRANSPORT IN SOD1^{G93A} MICE

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Background: Axonal transport is fundamental to the maintenance of normal neuronal function and deficits in transport are known to result in neuronal degeneration. Motoneurons appear particularly vulnerable to transport deficits and several mouse models with mutations in components of the transport system undergo motoneuron degeneration (1–3). Axonal transport is also disrupted in Amyotrophic Lateral Sclerosis (ALS) (4-5) with

retrograde transport defects even observed in embryonic SOD1^{G93A} motoneurons *in vitro (*6). However, whether these defects are an early feature of the disease that remains stable or whether they progress with disease remains unknown.

Objectives: We aimed to establish a dynamic *in vivo* assay that would allow the monitoring of retrograde transport in the intact sciatic nerve in anaesthetised mice. This assay would allow us to quantitatively analyse the characteristics of normal axonal transport in wild-type (WT) mice. Subsequently, changes in retrograde transport in SOD1^{G93A} mice *in vivo* at different disease stages could then be examined.

Methods: Alexa 555-TeNT Hc was injected into the tibialis anterior and gastrocnemius muscles of anaesthetised mice. Several hours later, the mice were re-anaesthetised and the sciatic nerve exposed. Following transfer to the microscope stage, retrograde transport was recorded in the intact sciatic nerve using time-lapse high-resolution microscopy.

Results: We have successfully established a reliable and reproducible *in vivo* assay to quantitatively monitor retrograde transport in the intact sciatic nerve. Analysis of SOD1^{G93A} mice revealed a significant deficit in retrograde transport at a presymptomatic stage (75 days) compared to WT mice. Analysis of the speed distribution profile in WT mice revealed three distinct components. However, in presymptomatic SOD1^{G93A} mice, the fastest component was significantly reduced, with a corresponding increase in the intermediate component.

Discussion: The results of this study reinforce previous evidence that indicates altered axonal transport in ALS (4,5). However, we have developed a dynamic assay that allows detailed analysis of transport characteristics including speed, processivity, frequency, pausing and changes in direction. Movement of multiple carriers can also be monitored. Moreover, this analysis can be performed at various disease stages. This novel assay therefore provides a method to closely examine and potentially dissect out the mechanisms underlying axonal transport defects in mouse models of ALS.

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SESSION 7B AUTONOMY AND QUALITY OF LIFE

C44 OREGON DEATH WITH DIGNITY ACT: IMPACT ON PATIENT CHOICE AND PALLIATIVE CARE PROVISION

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The Oregon Death with Dignity Act, which legalized physician-assisted suicide (PAS) for terminally ill patients, was enacted 10 years ago. This law allows a competent, requesting patient with an estimated life expectancy of less than six months, to receive a prescription for a lethal medication from a physician for the purposes of self-administration. Safeguards in the law require that a second physician must confirm the shortened life expectancy and the patient's competence; the patient must make one written and two oral requests over 15 days; and the patient must be aware of the availability of hospice care. If there is concern that a psychiatric disorder may be influencing the decision to hasten death, the patient must be evaluated by a mental health professional.

Support for the law varies among Oregon health professionals ranging from 40% of hospice chaplains and 51% of physicians, to 78% of psychologists. Only one third of Oregon physicians are willing to prescribe under the law. On the other hand, nine in ten Oregon hospice nurses who oppose the law would not actively oppose a client's choice for PAS.

Since enactment of the law, 292 patients have died by lethal medication, approximately 14/10,000 Oregon deaths. Patients who die by PAS are more likely to have a college education, and a scant 2% lack medical insurance. Although only 23 ALS patients have died by PAS, ALS is the disease associated with the highest likelihood of PAS; ALS patients are seven times more likely to use PAS compared to cancer patients. Over half of ALS patients in Oregon indicate they might consider legalized PAS, especially men, those who have high scores on measures of hopelessness, and those who are not religious. Studies of physicians and hospice workers who have cared for requesting patients, family members and patients themselves all point to the importance of staying in control, not being dependent on others, maintaining self-sufficiency and not burdening family as reasons patients pursue PAS. Depression and lack of social support were less commonly associated with PAS requests than predicted. Although 86% of patients who have died of PAS in Oregon are hospice enrolled, hospice referral and provision of non-judgemental support are the most effective interventions that may result in patients changing their mind about assisted suicide.

C45 THE UK MENTAL CAPACITY ACT AND MND – A LEAD FOR OTHERS TO FOLLOW?

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Background: The aim of the Mental Capacity Act is to ensure that best practice is followed when decisions have to be made for patients who are unable to express their wishes, either due to loss of competency or the inability to communicate.

Objectives: This paper looks at specific parts of the Act and how they impact on patients with MND and their carers. Comparisons are made with similar legislation/guidelines from other countries.

Methods: The Code of Practice informing implementation of the Act was studied to see how effective and practical it was in providing an ethical framework for resolving dilemmas in decision making for MND patients who are no longer able to express their wishes. Comparisons are made with legislation/guidelines from other countries.

Results, Discussion and Conclusion: The Mental Capacity Act provides a good framework for respecting the autonomy of patients who become unable to express their wishes when decisions have to be made on their behalf. Although some of the guidance is open to interpretation, the results of legal test cases will further inform future practice.

The Act would appear to be a major step forward in ensuring that best ethical principles are universally applied to decision making in these circumstances. This is in contrast to the wide variance which has existed in the UK until now and still exists in other parts of the world.

C46 FAMILY HEALTH CARE DECISION-MAKING AND SELF-EFFICACY WITH ALS PATIENTS AT THE END OF LIFE

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Background: People with ALS differ from those with other terminal illnesses in that they commonly retain capacity for decision making close to death. The role patients would opt to have their families play in decision-making at

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the end of life may therefore be unique. This study compared the preferences of patients with ALS for involving family in health care decisions at the end of life with the *actual* involvement reported by the family after death.

Objectives: The primary objective was to determine how patients preferred end of life decisions be made; whether independently, shared decision-making, or decision-making that is reliant on the family.

Methods: A descriptive correlational design with 16 patient-family member dyads was used. Quantitative findings were enriched with in-depth interviews of a subset of five family members following the patient's death.

Results: Eighty-seven percent of patients had issued an advance directive. Patients who would opt to make health care decisions independently (i.e., according to the patient's preferences alone), were most likely to have their families report that decisions were made in the style that the patient preferred. Those who preferred shared decision making with family or decision making that relied upon the family were more likely to have their families report that decisions were made in a style that was more independent than preferred. Family members described insufficient information about prognosis, great difficulty in decision making, and the fear of being blamed for the death of the patient by other family if they participated in the patient's decision to decline treatments.

Conclusions: The structure of advance directives may suggest to families that independent decision making is the ideal, causing them to avoid or underreport shared decision making. Fear of family recriminations may also cause family members to avoid or underreport shared decision making. Findings from this study might be used to guide clinicians in their discussions of treatments and health care decision making with people with ALS and their families.

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C47 PATIENT DECISION MAKING IN MOTOR NEURONE DISEASE & TUBE FEEDING: PILOT STUDY

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Background: Inability to swallow properly is one of the most obvious and distressing problems in motor neurone disease. Patients and carers must decide whether to use supplementary non-oral feeding by percutaneous endoscopic gastrostomy (PEG). The deliberations and timing of this decision are often haphazard. The optimum time for this choice, in terms of residual (quality) life expectancy and decision competency is unknown. Healthcare workers fear that even the offer of enteral feeding may be construed as an unwelcome landmark on the disease deterioration.

Objective: To identify preliminary issues that patients consider when deciding whether to have a PEG for supplementary feeding.

Methods: *Participants:* This qualitative pilot study involved 6 patients: 4 people (3f:1m) who decided to have a PEG and 2(f) who opted against it, aged 40–75 years. Time from MND onset: 4 months to 8 years. *Inclusion:* Patients with MND aged 18 years or over who have had to consider the insertion of a PEG or not. *Exclusion:* Patients with suspected cancer, or who have insufficient spoken or written English skills for the interview (although translation services would be an important element of a large scale study). *Interview:* Semi-structured interviews were conducted with each individual in a private room, when they attended regular MND clinic appointments. The interviewer was not part of the MND team. Relatives were allowed to be present if the patient wished, but were encouraged to allow the patient to speak for themselves. The researcher would repeat unclear responses to clarify for transcription and the repetition/interpretation was checked with the patient to ensure the patient's own words were used. Interviews were digitally recorded and transcribed verbatim. *Data analysis*: Emergent themes common to several participants and less frequent issues were identified.

Results: Patients were all from one clinic and the data reflect the experience with that team. All patients reported that they had enough information to inform their decision and that access to the MND team was open. There was variation in how much information people wanted – timing of this was important. Practical demonstration was appreciated but could be misinterpreted. Eating and drinking has many social aspects that are of equal or greater concern than just nutrition/hydration.

Discussion and conclusions: Our data show that the decision to have (or not) a PEG is a monumental step – having the operation may signal a feared move in the disease progression. Patients want information at different

rates – the health care team is perceived as most supportive when they appreciate this. Communication between primary and tertiary care is important. Patient perception of professional care should be assessed to ensure both parties have the same perspective. Participating in the pilot study allowed fresh discussion between family members about PEG related issues not raised previously.

C48 THE NEEDS & QUALITY OF LIFE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) FAMILY CAREGIVERS: A PILOT STUDY FROM CAREGIVERS PERSPECTIVE

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Objectives: The goals of this study were to develop a model of the needs of the Amyotrophic Lateral Sclerosis (ALS) family caregivers, to assess the quality of life of family caregivers, and to examine the relationship of identified needs and QoL to patient and family characteristics.

Method: This study was conducted in three phases: 1) ALS family caregivers (N=19) seen in a multidisciplinary ALS clinic were asked to identify their needs since the time of the ALS diagnosis of their family member, 2) The resulting 109 unique needs were printed on individual cards and sorted by 12 of the caregivers, 3) The needs were rated on importance by the same 12 caregivers who completed the sorting. The caregivers also completed a brief standardized measure of health-related quality of life (SF-8). The sort data were analyzed in two steps: 1) Nonmetric multidimensional scaling (MDS) and 2) Cluster analysis of the MDS values for each need. The SF-8 data was analyzed in relation to the needs ratings and patient and caregiver characteristics.

Results: The MDS produced an interpretable solution with a stress value of 0.36. The cluster analysis resulted in a four cluster map of ALS caregivers needs: Stage 1: Early Coping & Adjustment; Stage 2: Maintenance; Stage 3: Transition to End Stage; and Stage 4: Coping with Change & Loss. Results further indicated that the symptom presentation of ALS in the caregiver's family member was associated with lower levels of mental and physical health relative to national means, particularly for patients with initial bulbar symptoms ($p=0.03$). Additionally, caregivers who resided with their family member with ALS and were responsible for 24-hour care had poorer mental ($p=0.04$) and physical health ($p=0.02$) than ALS family caregivers that did not live with the ALS family member.

Discussion and conclusions: The data from this study of caregiver needs suggest that there is a predictable pattern of specific needs, and that QoL for caregivers is related to characteristics of the patient's disease, living arrangements and other individual differences. Future studies should examine the relationship of need satisfaction to QoL as well as the potential of caregiver support programs to meet identified needs and improve QoL.

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SESSION 7C CLINICAL ELECTROPHYSIOLOGY

C49 CORTICAL HYPEREXCITABILITY PRECEDES THE DEVELOPMENT OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background and objectives: Familial amyotrophic lateral sclerosis (FALS) is an inherited neurodegenerative disorder of the motor neurons in the cortex, brain stem and spinal cord, with 10–15% of cases attributed to mutations in the copper/zinc superoxide-dismutase-1 (SOD-1) gene. Although mutations in the SOD-1 gene result in toxic gain of function of the SOD-1 enzyme, the mechanisms underlying motor neuron loss remains unknown. The dying-forward hypothesis, in which cortico-motoneurons induce anterograde excitotoxic motoneuron degeneration, has been proposed as a potential mechanism. The present study applied novel threshold tracking transcranial magnetic stimulation (TMS) techniques in asymptomatic SOD-1 mutation carriers and clinically affected SOD-1 positive FALS patients to investigate the mechanisms underlying neurodegeneration in FALS.

Methods: Studies were undertaken in 17 asymptomatic carriers of the SOD-1 mutation followed longitudinally for three years. Results were compared to 6 clinically affected SOD-1 patients, 50 sporadic ALS patients and 55 normal controls. Threshold tracking TMS was performed using a 90 mm circular coil connected to a BiStim stimulator. Peripheral nerve excitability studies were performed in the same sitting, with responses recorded from the abductor pollicis brevis muscle.

Results: Short-interval intracortical inhibition (SICI) was significantly reduced in clinically affected SOD-1 FALS patients ($-1.2 \pm 0.6\%$) and sporadic ALS patients ($-0.7 \pm 0.3\%$) compared to the asymptomatic SOD-1 mutation carriers and controls (asymptomatic SOD-1 mutation carriers $8.3 \pm 1.8\%$; controls $8.5 \pm 1.0\%$, $P < 0.00001$). Reduction in SICI was accompanied by an increase in intracortical facilitation, motor evoked potential amplitude and magnetic stimulus-response curve gradient, all indicative of cortical hyperexcitability. In 14 asymptomatic SOD-1 carriers cortical excitability was normal. In 2 pre-symptomatic SOD-1 mutation carriers SICI was completely absent (SICI patient 1, -3.2%; patients 2, -1.3%), while in one patient there was a 32% reduction in SICI prior to symptom onset. These three individuals subsequently developed clinical features of ALS.

Discussion and conclusions: The present study confirms that cortical hyperexcitability develops before the onset of clinical features of ALS, thereby suggesting that cortical hyperexcitability underlies the basis of motor neurodegeneration in familial ALS.

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C50 DISTAL VS PROXIMAL MUSCLE INVOLVEMENT PATTERN IN PATIENTS WITH EARLY-STAGE AMYOTROPHIC LATERAL SCLEROSIS

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Background: Previous papers have suggested that in early-stage Amyotrophic Lateral Sclerosis (ALS) distal muscles are more affected than axial and proximal muscles.

Objectives: To evaluate the regional distribution of functional and electrophysiological abnormalities in patients with early-stage ALS.

Methods: Electromyography (EMG) and Nerve conduction studies (NCS) were performed in 45 sporadic ALS patients (15 with bulbar-onset and 30 with limb-onset symptoms) diagnosed according to El Escorial criteria. Mean time from onset of symptoms to diagnosis was 10.2 months. The functional rating scale (ALSFRS) was also evaluated.

Results: Fasciculation, fibrilation, PSW, reinervation potentials and decreased recruitment patterns were significantly more frequent and severe in distal limb muscles, especially in upper limbs, than in their corresponding axial and proximal muscles. The most affected items of the ALSFRS were those related to fine hand movements (handwriting, cutting food) and language. There was significant correlation between electrophysiological and functional involvement of hand muscles. Items related to gross movements (climbing stairs, walking, turning in bed) and dependent on proximal and axial muscles, were relatively preserved at this stage of the disease. Median nerve distal latencies (both motor and sensory) and motor conduction velocity (elbow-wrist) were affected in 52% of the patients, despite normal amplitudes of the responses.

Conclusion: Our data suggest that in early stages of ALS, intrinsic hand muscles are selectively affected in comparison to more proximal limb or axial muscles. These results

are consistent with a regional increased vulnerability of the motor neuron pools related to these muscles. Probable pathogenic mechanisms responsible for these differences are discussed in the light of the currently most accepted theories linked to ALS.

C51 DENERVATION-REINNERVATION OF AXIAL AND DISTAL SEGMENTS IN ALS: CORRELATION WITH DIAPHRAGM INVOLVEMENT

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Background: In ALS, degeneration of long axons in peripheral motor nerves has been regarded as a 'proximal-distal axonopathy', due to a primary pathological process in anterior horn cells. This concept is testable by comparing proximal and distal loss of motor units in the same myotome.

Objectives: To compare denervation-reinnervation between proximal and distal muscles of the same myotome in ALS, and to examine the relation between paraspinal cervical and limb muscle denervation, and paraspinal and diaphragmatic denervation.

Methods: We used concentric needle EMG to study denervation (fibrillation and positive sharp-waves, fibs-sw) and reinnervation (multiMUP analysis) in a paraspinal and a limb muscle innervated by the C6 and L5 segments in 32 patients with ALS and 46 control subjects. All patients had an ALS-FRS score >29. In all limb muscles studied the MRC graded strength was >3. One side was investigated in each patient. In addition, we considered whether diaphragmatic denervation (fibs-sw) was more severe in patients with cervical paraspinal (short axon) or biceps (long axon) denervation.

Results: In controls, distal and proximal muscles were normal. In ALS we found no significant differences between MUP analysis in paraspinal and limb muscles in these two segments ($p>0.05$), except for a higher MUP amplitude in tibialis anterior ($p<<0.001$). Fibs-sw were most frequent in tibialis anterior ($p<0.01$), a muscle which showed more unstable motor units than the homologous paraspinal muscles. Denervation of the diaphragm was similar in patients with cervical paraspinal denervation ($p=0.3$) and biceps denervation ($p=0.4$).

Discussion and conclusions: These results are consistent with similar and random involvement of motor neurons in different motor neuronal pools which innervate individual spinal segments. This is evidence refuting the concept of proximal-distal axonopathy in ALS. However, distally predominant fibs-sw represent susceptibility to ongoing denervation in reinnervated distal axons, as mirrored by the more severe instability of these distal motor units. This suggests that motor neurons with very long axons experience additional stress in maintaining axoplasmic transport over time. Initial successful reinnervation is followed by a more ongoing denervation in these long axon motor units than in shorter axon units.
Diaphragmatic denervation does not correlate with other cervical motor neuron involvement, indicating functional autonomy in the phrenic nerve nuclei, located anatomically in a different position in the cervical anterior horn.
As an additional finding, we emphasize the value of paraspinal muscle EMG in the clinical diagnosis of ALS, in particular in the differential diagnosis with axonal motor neuropathies.

C52 CONTINUOUS AMBULATORY DIAPHRAGM EMG MEASUREMENTS: ASSESSING RESPIRATORY CONTROL AND FUNCTION UTILIZING THE DIAPHRAGM PACING STIMULATION (DPS) SYSTEM

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Background: Diaphragm contraction is necessary for ventilation although respiratory control is incompletely understood. Important structures likely include: the special somatic respiratory nuclei of the brainstem, the cerebral cortex and the carotid body among others. Output from these centers is transmitted through the phrenic nerve to the diaphragm but muscle electrical activity cannot be easily measured further limiting investigations of neuromuscular control of breathing. Ongoing work from our group has led to the development of intramuscular electrode pacing of the diaphragm in patients with respiratory failure. An interesting by-product of this research is to have a continuously available electrode in the diaphragm that could theoretically be used to monitor EMG activity.

Objective: This report analyzes initial feasibility and applications of continuous EMG assessment of the diaphragm in ALS/MND patients implanted with the diaphragm pacing stimulation (DPS) system (Synapse Biomedical, Ohio).

Methods: Amyotrophic lateral sclerosis (ALS) patients who were implanted with electrodes at the motor point with DPS system were analyzed utilizing a home polysomnography system (CleveMed, Ohio). Continuous EMG was recorded from the implanted diaphragm electrodes.

Results: Four implanted patients were studied. The ALS patients studied showed that diaphragms with less volitional or stimulated movement under fluoroscopy also had a reduction in the generated motor response demon-

strated by the EMG tracings. Diaphragm EMG activity correlated with the appropriate thoracic and abdominal movements during sleep. Stronger EMG activity correlated with larger chest and abdomen movements along with visualized diaphragm movement under fluoroscopy. Sleep EMG obtained in one ALS patient receiving non-invasive positive pressure ventilation (NIPPV), showed significant periods of absent diaphragm EMG activity which led to lower subsequent SpO2 which in turn led to increased EMG activity. The home based study in this patient showed unsuspected oxygen desaturations 33% of the time which necessitated changes in NIPPV. Another patient showed that decreasing rhythmic EMG activity and decreasing O2 saturations caused a subsequent increased EMG activity that corrected the desaturation. Correction of O2 desaturations in the second patient with NIPPV resulted in a stable but decreased diaphragm EMG activity.

Conclusions: We show the initial feasibility of continuous EMG assessment of the diaphragm in ALS/MND patients implanted with the DPS system and its use in analysis of sleep dysfunction. EMG activity correlated with progression of disease. Because the electrodes are implanted at a standardized point (diaphragm motor point - the point where stimulation causes maximal contraction), serial diaphragm EMGs may be a viable biomarker for disease progression. The ability to follow serially with EMG at a well described reproducible motor point the most important muscle of respiration may also allow rapid assessment of new therapies. Also as other directed therapies are developed, the ability to utilize the bilateral DPS system to monitor unilateral or bilateral therapy and then help with ventilation when needed may hold promise.

C53 RELIABILITY OF A STANDARDIZED FORM OF MODIFIED MULTIPOINT MUNE

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Background: Motor unit number estimation (MUNE) is an electrophysiological technique that directly assesses nerve fiber loss. MUNE has been successfully used to detect motor neuron loss in ALS prior to symptom onset, in the longitudinal evaluation of ALS patients and as a predictor of survival in ALS. Use of MUNE has been reported in two multicenter clinical trials; in both trials the statistical method was employed. Although values obtained were reliable and declined with disease progression, motor unit instability limits the utility of this technique. Our goal is to develop a rapidly performed MUNE method that has good test-retest reliability and can be well standardized. We therefore have devised a modification of multipoint and incremental MUNE, which uses standardized stimulus locations and limits the numbers of increments recorded at each site.

Objectives: To obtain measures of intrarater reliability for modified multipoint MUNE, in preparation for longitudinal studies on patients with ALS.

Methods: Median nerve MUNE was performed recording from abductor pollicis brevis in 10 normal control subjects. Recordings were made using standard recording montages with self adhesive electrodes. Each nerve was stimulated at 3 standard locations; 6 cm proximal to the recording electrode, 4 cm proximal to position 1, and in the cubital fossa at the level of the epicondyles. At the distal location, a maximum compound motor action potential (CMAP) was recorded. At each location, using weak stimuli with fine stimulus control, 3 incremental responses were recorded. An estimate of the average single motor unit potential (SMUP) amplitude was calculated by summing the 3rd response at each site and dividing by 9. MUNE is calculated by dividing the average SMUP into the CMAP. For each subject, two trials were performed by the same examiner on different days (interval 1–7 days).

Results: For 10 normal subjects ranging in age from 24–54 years, MUNE averaged 210, with a standard deviation of 60. Intrarater reliability was 10.1%. The correlation coefficient comparing first to second evaluation was 0.91.

Conclusions: The interrater reliability for the modified multipoint MUNE technique compares favourably with that of other reported MUNE methods. The technique can be performed quickly, on any standard EMG machine. Further studies will evaluate interrater reliability and assess longitudinal changes in patients with ALS.

C54 REVISED ELECTRODIAGNOSTIC CRITERIA FOR EARLY DIAGNOSIS OF ALS

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Introduction: Current electrophysiological criteria used in the diagnosis of ALS are relatively rigid and insensitive relative to the level of clinical suspicion. A consensus group of 11 neurologists/clinical neurophysiologists sponsored by the International Federation of Clinical Neurophysiology, met in Awaji-shima, Japan in December 2006 to consider this issue.

Objectives: To describe electrophysiological criteria to facilitate early diagnosis of ALS, in relation to, and not independent of, clinical criteria.

Methods: An evidence-based approach was used, involving review of the literature in relation to defined topics in the clinical neurophysiology of ALS, in order to consider the value of existing and new technologies in the diagnosis of ALS. Relevant issues were addressed by each of the 11 participants.

Results: It was concluded that the current framework for clinical diagnosis, based on levels of certainty, as set out in the Airlie House modification of the El Escorial document should be respected. We recommend two major changes to the criteria respecting electrophysiological abnormalities in the diagnosis of ALS: 1) We regard significant neurogenic

abnormalities in concentric needle EMG (chronic partial denervation), taken with clinical features in another muscle of different root and nerve innervation, as sufficient abnormalities in a single limb to class as involvement in one spinal region. This change will render the category ''Lab-supported probable ALS'' superfluous. 2) In ALS, muscles may show EMG evidence of chronic neurogenic change in the absence of fibrillations or positive sharp waves (fibs-sw). We consider that the presence of fasciculation potentials (FPs) in a muscle showing chronic reinnervation by motor unit potential (MUP) criteria, especially MUPs of complex and unstable morphology, constitute evidence of chronic neurogenic change, even in the absence of fibs-sw. FPs in this situation are often of complex morphology, indicating their origin in reinnervated units. 3) Other positive and negative features required for the diagnosis of ALS as set out in the Airlie House criteria should be carefully followed.

Discussion: These changes are in accord with clinical experience and with data in the literature. They will facilitate earlier diagnosis of ALS. They recognise the clinical value of complex remodelled MUPs, and of the characteristic occurrence of FPs in chronic partial reinnervation in ALS. This is important in clinical practice and also in recruitment into clinical trials. We propose testing these criteria in a prospective evaluation. Retrospective evaluation in a database suggests they will have utility.

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SESSION 8A PROTEIN MISFOLDING AND AGGREGATION

C55 CAUSES AND CONSEQUENCES OF PROTEIN MISFOLDING IN NEURODEGENERATIVE DISEASE

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Abstract not available.

C56 AMYOTROPHIC LATERAL SCLEROSIS-LINKED PRO56SER VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN IMPAIRS UNFOLDED PROTEIN RESPONSE

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Background: The Pro56Ser (P56S) mutation in vesicle-associated membrane protein-associated protein B (VAPB) is a recently identified ALS-linked gene (ALS8) and it causes autosomal-dominant motoneuronal diseases (MNDs) (1). We recently reported that P56S-VAPB is a highly insoluble mutant (2). Furthermore, we found that overexpression of wt-VAPB triggers splicing of XBP1-VENUS, which is an indicator of unfolded protein response (UPR) (2).

Objectives: We investigate the pathomechanism of P56S-VAPB-linked ALS to identify the common mechanism of ALS.

Methods: To investigate how the P56S-VAPB works *in vivo*, we used a yeast knockout strain lacking Scs2 (yeast homolog of VAPB). UPR induction is confirmed by luciferase assays and immunoblot analyses. The motoneuronal cell-viablity assay was performed with WST-8 assay and the cell death assay was performed with LDH assay. VAPB degradation assay was performed with cycloheximide in the presence or absence of a proteasome inhibitor.

Cell fractionation assays were performed with sucrose-gradient ultracentrifugation and iodixanol-gradient ultracentrifugation.

Results: Fractionation analyses and immunocytochemical analyses clarified that P56S-VAPB makes aggregates partially localizing in endoplasmic reticulum (ER). We also demonstrate that P56S-VAPB is a non-functional mutant *in vivo* by luciferase assays and yeast growth assay. Furthermore, P56S-VAPB attenuates UPR and causes ER stress vulnerability by enhancing degradation of wt-VAPB. Downregulation of endogenous VAPB by small interfering RNA also impairs UPR and causes ER-stress vulnerability.

Discussion and conclusion: We demonstrate that P56S-VAPB that is a non-functional mutant *in vivo* attenuates UPR and causes ER stress vulnerability by enhancing degradation of wt-VAPB. Combined with the observation that downregulation of endogenous VAPB by small interfering RNA also impairs UPR and causes ER-stress vulnerability, loss of the VAPB function by a point mutation in VAPB gene contributes to ER stress-vulnerability of motor neurons related to ALS-pathogenesis.

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C57 PROTEIN AGGREGATION AND THERMODYNAMIC STABILITY ARE RISK FACTORS IN ALS PATIENT SURVIVAL

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Background: Protein aggregation is a pathological hallmark of many neurodegenerative diseases, including ALS. Whether aggregation is a cause or a consequence of disease is fiercely debated, and this debate is fuelled by evidence both for and against the toxicity of protein aggregates (1). Protein aggregation can result from intrinsic factors such as changes in protein thermodynamic stability, charge, the propensity to form α-helices and β-sheets, and hydrophobicity. Recent studies have developed algorithms that predict how a given mutation affects protein aggregation rates (2,3) and have demonstrated that in vitro protein aggregation rates depend upon physicochemical properties, specifically changes in charge, hydrophobicity, and secondary structure.

Objectives: To identify risk factors for fALS patient survival.

Methods: Recent studies have developed algorithms that can predict how a given mutation will change the rate of protein aggregation. We applied these algorithms to fALS-causing SOD-1 mutations. We also combined all published thermodynamic stability data of fALS SOD-1 mutants.

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Results: Application of the algorithms to fALS-causing SOD-1 mutations demonstrated that SOD-1 aggregation rate is a factor in ALS patient survival. We also showed that loss of protein thermodynamic stability is a factor in ALS patient survival. These results account for 70% of the variability in fALS patient survival times, as well as general aspects of the neurodegenerative diseases, including late disease onset and the selective vulnerability of particular neurons. Finally, we fitted Kaplan-Meier survival cures of fALS patients using an equation that is designed to fit in vitro protein aggregation data, and the lowest R^2 we observed was 0.80, providing a striking example of how no ALS patient (of the hundreds used in our study) survives the point at which 100% of mutant SOD-1 is predicted to aggregate.

Discussion: A mechanism of neurodegeneration that includes protein aggregation predicts the following aspect of disease: 1) delayed onset; 2) cells with the highest concentration of the aggregation-prone protein are preferentially vulnerable; 3) the rate of protein aggregation affects the survival time of patients following disease onset. Each of these is observed in fALS, and delayed onset is common in the neurodegenerative diseases. Our method of predicting patient survival times from basic physicochemical changes to proteins is also applicable to, and has implications for, sporadic ALS.

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C58 SOLUBLE MISFOLDED SOD1 SPECIES ENRICHED IN SPINAL CORDS OF TRANSGENIC MURINE ALS MODELS

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Background: Mutants of superoxide dismutase-1 (SOD1) cause amyotrophic lateral sclerosis (ALS) by an unidentified cytotoxic mechanism. We have previously shown that the stable human (h) SOD1 mutants D90A and G93A are abundant and show highest levels in liver and kidney in transgenic murine ALS models, while the unstable G85R and G127insTGGG (G127X) mutants are scarce but enriched in the CNS. These data indicated that minute amounts of misfolded SOD1 enriched in the motor areas might exert the ALS-causing cytotoxicity.

Objectives: To determine amounts and analyse the structure of soluble misfolded hSOD1 species in tissues of murine transgenic ALS models.

Methods: Antibodies specific for misfolded hSOD1 were generated using immunization peptides in the sequence of the enzyme. These were used in an immunocapture protocol for analysis of misfolded hSOD1 in 20,000g supernatants of murine tissue homogenates. A hydrophobic interaction chromatography protocol was also developed and used for that purpose.

Results: All G127X and the major part of the G85R hSOD1s bound in the assays, but only minute subfractions of the G93A and D90A mutants. Wild-type hSOD1 bound even less. The absolute levels of misfolded hSOD1 were, however, similar in the murine ALS models and they were enriched in the susceptible spinal cord. This enrichment was seen from birth until death, and the levels of soluble misfolded hSOD1 were comparable to the amounts of hSOD1 that become sequestered in aggregates in the terminal stage. The misfolded hSOD1 was composed of disulfide-reduced subunits lacking metal ions, and also subunits that apparently carried non-native intrasubunit disulfide bonds. Misfolded hSOD1, released from the antibodies by the peptides used for immunization, was shown by gel chromatography to be composed of monomers, trimers and oligomers.

Discussion: The soluble misfolded hSOD1 species will expose sticky hydrophobic internal structures which might interact with essential cellular factors in ways that cause cytotoxicity. They form a least common denominator amongst hSOD1 mutants with widely different molecular characteristics, and are thus potential culprits for the cytotoxicity that causes ALS. The susceptibility of the motor areas of the CNS may be caused by an inadequate ability to recognize and degrade misfolded SOD1 species.

C59 DETECTION OF MISFOLDED SOD1 IN SPORADIC AND FAMILIAL ALS

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Background: Protein misfolding diseases result from the toxicity associated with the conversion of the native state of a protein into a pathologically misfolded conformation induced by mutation and/or environmental triggers. In 20% of familial amyotrophic lateral sclerosis (ALS) mutations in superoxide dismutase (SOD1) cause the protein to misfold and form intracellular inclusions.

Toxicity of these cytoplasmic aggregates is thought to arise from aberrant interactions with the protein-folding chaperone system or from inhibition of proteasomes. Toxicity has also been proposed to result from aberrant interactions with mitochondrial proteins such as Tom20 or Bcl-2 because SOD1 has been detected in mitochondria from the spinal cord and brain, and mitochondrial vacuolization is an early event in ALS models.

Objective: The ability to detect misfolded proteins *in vivo* would be very beneficial for diagnosis and for furthering research into the molecular basis of the disease. Our approach to developing *in vivo* conformational probes involves generation of antibodies that detect non-native states of a protein, yet do not bind natively folded molecules.

Methods: We have developed an antibody that specifically recognizes monomer/misfolded forms of SOD1. This antibody was raised to an epitope within the SOD1 dimer interface, which is normally buried within the native obligate homodimer. This SOD1 exposed dimer interface (SEDI) antibody, only recognizes SOD1 conformations where the native dimer is disrupted/misfolded, exposing the hydrophobic dimer interface. We have used this antibody to detect misfolded SOD1 in transgenic rodent ALS models and in human tissue.

Results and discussion: Using the SEDI antibody we establish the presence of monomer/misfolded SOD1 in three ALS mouse models, with G37R, G85R or G93A - SOD1 mutations, and in a human individual with an A4V SOD1 mutation. Despite ubiquitous expression, misfolded SOD1 is found primarily within degenerating motor neurons and is preferentially enriched in mitochondrial and membrane fractions from affected spinal cords. Misfolded SOD1 appears before symptom onset and decreases at disease end-stage, concomitant with motor neuron loss. Extracellular misfolded SOD1 was also observed by exclusion of double staining with markers of CNS cell types (anti-GFAP, anti-Mac2). Misfolded SOD1 was present in brain and spinal cord, but absent in tissues that are unaffected by disease: liver, heart, muscle, and kidney. Spinal cord sections from human individuals with sporadic ALS and familial ALS with and without SOD1 mutations are being examined.

C60 DECONVOLUTION 3-D IMAGING OF TDP-43 INCLUSIONS IN ALS: UNCOVERING A CONTINUUM IN ALS PATHOLOGY

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Background: The nuclear factor TAR DNA-binding protein (TDP-43) has recently been identified as a component of neuronal cytoplasmic ubiquitinated inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis (ALS). In ALS, three distinct types of intraneuronal ubiquitinated inclusions have been described, skein-like inclusions, round inclusions and hyaline conglomerate inclusions (HCIs). HCI's appear to be specific to cases carrying SOD1 mutations and are TDP-43 negative. In contrast skein-like inclusions and round inclusions are common to both familial and sporadic ALS and are TDP-43 positive. The relationship between these types of inclusions is unknown.

Objectives: Here we have used 3D-deconvolution imaging of skein-like inclusions and round inclusions fluorescently-labeled with antibodies to ubiquitin and TDP-43 or ubiquitin, TDP-43 and peripherin, respectively to obtain greater detail of the molecular structure of these types of ubiquitinated inclusions in ALS.

Methods: Formalin-fixed, paraffin embedded sections (6μm) from the lumbar spinal cord of eight sALS cases were fluorescently double labeled with antibodies to TDP-43 and ubiquitin or triple labeled with antibodies to TDP-43, ubiquitin and peripherin. Images were captured as Z-stacks using the 63x oil objective of a Leica DMI-6000 digital microscope, then deconvoluted and reconstructed in 3-D using Volocity imaging software (Improvision).

Results: Spectacular images were obtained of skein-like inclusions and round inclusions using 3D-imaging that are not achievable using conventional 2D approaches. We have found that, instead of being distinct entities, skein-like inclusions and round inclusions are in fact a continuum of the same underlying pathology involving TDP-43, with skein-like inclusions appearing to wrap around and condense, culminating in the formation of round inclusions. Interestingly, although TDP-43 is clearly associated with ubiquitin in the skein-like inclusions, round inclusions are comprised of a central core of non-TDP-43 immunoreactive ubiquitinated material that is surrounded by a halo of TDP-43 immunoreactivity. These findings indicate that TDP-43, in contrast to published findings, is not the only major ubiquitinated protein that contributes to the formation of round inclusions in ALS. Furthermore, we consistently observed an absence of labeling in round inclusions between the central core of ubiquitin and the surrounding TDP-43 halo. Using triple labeling, we show that this gap is filled by peripherin and provide a movie to emphasize this point.

Conclusions: Using 3D imaging of skein-like inclusions and round inclusions fluorescently labeled with TDP-43, ubiquitin and peripherin antibodies, we have obtained remarkable detail as to the structure of these types of inclusions in ALS. Firstly, we show that skein-like inclusions and round inclusions are in fact a spectrum of the same pathology involving TDP-43. Secondly, TDP-43 is not the only major ubiquitinated protein in round inclusions. Thirdly, peripherin, although not ubiquitinated, is an integral component of round inclusions and only infrequently associated with skein-like inclusions.

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SESSION 8B LEARNING FROM THE EXPERIENCE OF PEOPLE LIVING WITH ALS/MND

C61 PERSONAL NARRATIVES OF LIVING WITH MOTOR NEURONE DISEASE – A SEARCH FOR NORMALITY

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Background: This paper reports findings from a qualitative study for the MND Association of England, Wales and Northern Ireland. The shock of diagnosis of ALS/MND is followed by prolonged uncertainty about how the condition will progress and how quickly. Adapting to this new situation is a practical and emotional challenge explored in this research. The study will also lead to a new freely accessible website where users can see and hear people with ALS/MND talk about their experiences. The aim is both to inform and support others with the condition and their carers, and to offer a teaching resource for health professionals to gain greater insight into personal perspectives on living with ALS/MND.

Objectives: To understand through personal narratives how different people respond to a diagnosis of ALS/MND; the impact on themselves and their family; and the strategies they may use to re-establish a sense of control and normality in their lives. To develop a new ALS/MND area of the www.dipex.org site (personal experiences of health and illness).

Methods: A narrative interview study with a maximum variation sample of ≈40 people diagnosed with ALS/MND across the UK. Interviews take place in the person's home. They are video or audio-recorded and transcribed verbatim. Participants review their transcript before agreeing to its use. Analysis will incorporate both thematic and narrative analysis, using N6 qualitative data analysis software.

Findings: Participants describe an often lengthy search for diagnosis. The shock of eventually learning they have ALS/MND may be tinged with relief that they at last know what they are dealing with. Immediately after diagnosis people often report a feeling that life is already over, and there is no point planning ahead or looking forward even to short-term events or milestones. Others describe an immediate instinct to deny or resist the condition. Over time, many people report engaging in a process of re-establishing a sense of normality and regaining control and purpose in their lives. Because ALS/MND often progresses rapidly, this is not simply a one-off adjustment, but a repeated cycle of facing each new symptom phase and incorporating it into 'normal' daily life. Strategies to remain mobile feature prominently, and the sense of freedom and normality afforded by driving is of particular importance. Holidays are also identified as a key way of maintaining social participation. Trying to keep doing the 'normal' things – whether this is work, talking, household tasks, leisure activities or personal hygiene – may give way to a revised sense of what is 'normal', and a search for practical alternative ways to do things, or different things to do.

Discussion: The paper will conclude by discussing the relevance of biographical disruption (1) to ALS/MND, a theory usually applied to chronic conditions. Findings suggest diagnosis does indeed mark a point of biographical disruption, or even abruption and despair. However, whilst some people struggle to emerge from this, many describe what could be described as biographical repair or narrative reconstruction (2), as they find normality wherever they can and make sense of their new circumstances.

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C62 TELESOCIAL MEDICINE FOR ALS/MND – PATIENTSLIKEME.COM

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Background: Over the past decade, an increasing number of patients have used the internet as a static source of information about their condition. Patients with neurological conditions such as motor neurone disease, Parkinson's disease, multiple sclerosis, and epilepsy are forming online communities based around interactive message boards or "forums". These allow patients throughout the world to share information and personal experiences in a "safe" virtual environment, usually through sites set up by hospitals, charities or individual patients. Since 2004 a second wave of mainstream internet sites such as EBay, Flickr, MySpace, YouTube and Wikipedia have been launched, emphasising collaborative information sharing, under the title "Web 2.0". Here we report a new website for patients with neurological conditions which allows them to share their experiences in the traditional way, but in addition contains sophisticated features for tracking their symptom severity, medication usage, and use of assistive technology.

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Objective: The aims are threefold; first to allow users to find a ''Patient like me'' to ask questions or share advice; secondly to empower patients to take a lead in managing their own condition by equipping them with tools to measure their symptom severity and highlight their needs during the neurological consultation; thirdly as a platform for research to rapidly access thousands of patients to conduct online studies.

Methods: We have established a community of 1,000+ people with ALS (PALS), 500+ caregivers (CALS), and 400+ guests (doctors, researchers, non-profit staff). It is free for all members to use. Patients complete their diagnostic history and are able to track their progression using a self-report version of the ALSFRS. Patients can print off their profiles as ''doctor visit sheets'' to quickly encapsulate their progress as well as monitor any changes in symptom severity. They are also able to network socially in order to swap views, information, and practical tips. We have recently developed a fully-featured research module which allows us to carry out surveys and develop new instruments.

Results: Feedback has been overwhelmingly positive, with many patients reporting that the site helps empower them to manage their own care better. The site is also used as a source of emotional support and for socialising. Research projects have included surveys on cognitive dysfunction, excessive yawning as a possible manifestation of emotional lability, and an extension to the ALSFRS-R scale designed to measure the progress of patients whose physical function falls below floor level on the existing scale.

Conclusions: PatientsLikeMe represents a powerful way of harnessing collective knowledge and enabling patients and carers to make decisions about their healthcare. It also represents an unparalleled platform for research in patients with rare conditions. Two more communities have recently been launched in MS and Parkinson's disease.

C63 FROM SYMPTOM AWARENESS TO ILLNESS RECOGNITION AND BEYOND: PERSONAL EXPERIENCES OF BEING DIAGNOSED WITH ALS/MND

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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 a) becoming aware of symptoms b) the recognition of these as indicative of illness serious enough to warrant medical attention and c) the process of being diagnosed with ALS/MND.

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 with reference to Frank's narrative typologies, *restitution, chaos and quest* (2). Data management was aided by Nvivo 7 software.

Results: A number of key themes emerged. This paper focuses on the story of the time leading up to and around the diagnosis. Authors reveal how they recognise abnormalities presenting as symptoms, attribute changes in their body to illness and assess the magnitude of their condition. This results in a change of status from *symptom awareness* to *illness recognition*. Adopting the characteristics of the restitution narrative numerous texts reveal authors' reactions to vague initial symptoms and their attempts to *explain away* the problem as being of little significance. The realisation that their condition is serious is often reflected in a move away from a restitution narrative towards one of chaos, with its dominant message of loss of control. Many authors describe *sanctioning* of help-seeking where their health concerns are *legitimated* by others. Undergoing investigative tests is consistently described as an unpleasant, confusing and often *dehumanising* process. Authors recount trauma-filled reactions when given their diagnosis. Many react in *disbelief* that their condition can be so serious, yet they feel remarkably well and display only minor symptoms. Some report feelings of *anger*, *disappointment* and *abandonment* resulting from the inability of medical science to offer a viable cure. Seeking recovery, often through the use of alternative treatments, provides evidence of a return to the restitution narrative, while viewing the illness as a challenge to overcome reflects the adoption of the quest narrative.

Discussion and conclusion: These illness narratives throw light on the personal experiences leading up to and around the time of a diagnosis of ALS/MND. The results have implications for the training of health care personnel and for the care provided at this distressing and traumatic time.

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C64 IDENTIFYING HOW PEOPLE WITH MND/ALS TALK ABOUT LIVING THROUGH THEIR ILLNESS: A NARRATIVE STUDY TO ENHANCE SELF-MANAGEMENT

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Background: There is little known about how people live or manage coping with motor neurone disease or amyotrophic lateral sclerosis, (MND/ALS) which is essential knowledge for developing approaches to enhance quality of life and patient self-management. This study used narrative research methods to explore how patients with MND/ALS talk about living and coping with this disease. Narrative research, whereby people who are ill are encouraged to tell their stories, can elucidate issues previously overlooked in biomedical approaches to disease management. One approach to classifying illness narratives involves identification of storylines or 'plots'. This paper presents four 'plots' which were identified and developed into a framework of narrative styles which may be helpful for patients, family care-givers and professionals to enhance quality of care and patient self-management.

Objectives: 1) To explore patient experiences of living and coping through MND/ALS; 2) to ascertain how people talk about living and coping with MND/ALS

Methods: The study involved a series of longitudinal narrative case studies. Thirteen patients were recruited through purposeful sampling. Six rounds of in-depth narrative interviews were conducted every three months over an eighteen month period. A narrative thematic framework approach (1,2) was developed which takes into account an entire story and focuses on its form to identify narrative themes. This approach is responsive to emergent issues allowing construction and revision of the themes and the developing framework as new and emergent themes are noted and added. Results are summarised in a final thematic frame and also presented with illustrative quotations relating to the themes.

Results: A framework of four narrative styles was identified and the styles named fracturing, sustaining, preserving and enduring. Some participants presented a kaleidoscopic interchange of the four narrative styles whereas others presented a consistent style throughout their experience.

Discussion: Identification of patient narrative styles offers unique insight into patients' approaches and responses to coping with MND/ALS. It is suggested that knowledge of the narrative styles may be helpful for patients, family care-givers and professionals as a framework for understanding patient approaches to living with a life-limiting illness which may enhance patient self-management and quality of life.

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SESSION 8C CLINICAL GENETICS AND BIOMARKERS

C65 LONGITUDINAL METABOLOMIC PROFILING IN PLASMA FROM PARTICIPANTS WITH ALS

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Background: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease in adults. The mechanisms underlying disease onset and progression are unclear. Biological markers of disease progression in ALS are lacking. In our previous metabolomic studies several compounds were identified in plasma from people with ALS at higher or lower levels relative to healthy control individuals. The level of several of those metabolites decreased as forced vital capacity (FVC) decreased, indicating that these compounds may be candidate markers for disease progression. In this study we evaluated changes in metabolites in plasma samples from participants with ALS that were collected over a 12 month time period.

Objectives: The study objective was to identify significant metabolites associated with disease progression in ALS.

Methods: Metabolomic analysis was performed on plasma samples from 63 participants with ALS enrolled in a multicenter clinical trial of topiramate and who were assigned to the placebo arm. Plasma samples were collected from each participant at the screening, 1 month, 6 month, and 12 month visits. Institutional Review Board approval was obtained for the secondary use of these samples for metabolomic analysis. Following LC-MS and GC-MS analysis, the metabolomic data was curated and then analyzed statistically to identify metabolites that are associated with disease progression. Information on baseline characteristics and clinical measures of the rate of disease progression were available on all subjects. These include measurements of quantitative muscle strength testing, forced vital capacity and the ALS functional rating scale (ALSFRS). Statistical analyses were carried out to identify metabolites that change with time and to identify metabolites that change with clinical measure of disease progression.

Results: We identified specific metabolites that correlated with disease progression. Named metabolites that correlated with change in ALSFRS included arginine, 4-guanidinobutanoic acid, gamma-glu-leu, (p-hydroxy)phenyllactate, inositol-1-phosphate, mannose and creatinine. Named metabolites that correlated with change in FVC included creatinine, glutamic acid, aspartate,
3-methyl-L-histidine, 4-guanidinobutanoic acid, para-xanthine gamma-glu-leu. Four metabolites, two named and two unnamed, change with both ALSFRS and FVC. These metabolites are creatinine, 4-guanidobutanoic acid and the unnamed compounds X-1988 and X-3073. Several of the named metabolites (glutamic acid, arginine, (p-hydroxyphenyl)lactic acid, creatinine) were identified as significantly different in our earlier studies comparing ALS patients with healthy control subjects.

Conclusions: We identified several metabolites that change over time or are correlated with the clinical measures of progression. A number of interesting compounds were identified, several of which are consistent with earlier studies that compared subjects with ALS and healthy controls. This suggests that metabolomic analysis can identify biomarkers correlated with ALS disease progression.

C66 CHARACTERIZATION OF THE CSF PROTEOME BY MASS SPECTROMETRY TO DISCOVER PEPTIDE BIOMARKERS FOR ALS

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Background: Recent studies have used mass spectrometry based proteomics of the cerebrospinal fluid (CSF) or blood plasma to discover potential biomarkers for ALS. Many of these studies have utilized proteomic technologies that either focus on abundant proteins or fail to identify the protein peak that corresponds to a putative biomarker. A more detailed and comprehensive examination of the CSF proteome is required to both compile a list of proteins present in this biofluid and to compare peptide and protein modifications that distinguish ALS from control subjects.

Objectives: To perform a comprehensive analysis of the CSF proteome in ALS and control subjects to discover protein based peptide biomarkers for ALS.

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Methods: CSF from 60 ALS, 40 healthy control, and 40 Alzheimer's disease subjects were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). We first pooled aliquots from 10 individual CSF samples within each subject group prior to depletion of the 12 most abundant proteins and trypsin digestion. Therefore we generated multiple pooled samples of ALS and control CSF samples. Tryptic peptides were then analyzed by Thermo LTQ XL linear ion trap mass spectrometer. All samples were analyzed in triplicate and all peptides were sequenced by tandem mass spectrometry to identify proteins present in the CSF and post-translational modifications within each peptide. Data were analyzed by Thermo BioWorks 3.3.1 and SIEVE automated label-free differential expression software.

Results: We characterized tryptic peptides present in the CSF of ALS and control subjects. Over 1,000 proteins were identified in the CSF proteome. By comparing the tryptic peptide products for each protein, we determined specific peptides present only in ALS or control subjects. Some of these peptides also exhibit post-translational modifications specific to either control or ALS subjects. We have determined that peptides derived from multiple proteins including chromagranin B, SPARC-like protein 1, BASP1, Ceruloplasmin, Clusterin, Angiotensinogen, Complement C1 and two distinct Insulin-like growth factor binding proteins exhibit specific alterations in the CSF of ALS patients.

Discussion/Conclusions: High performance LC-MS/MS provides a platform to identify protein derived peptides present in the CSF and determine protein and peptide alterations that occur in ALS patients. We have identified over 1,000 proteins in the CSF proteome. We identified multiple proteins altered in ALS patients that participate in cell adhesion, inflammation, and vesicle transport pathways, in addition to proteins of the neuroendocrine and renin-angiotensinogen systems. These results suggest that quantification of specific peptides within the CSF may aid in the diagnosis of ALS and reveal novel therapeutic targets.

C67 MUSCLE PROTEOMIC PROFILE IN THE TRANSGENIC SOD1G93A MOUSE AND IN ALS PATIENTS

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Background: ALS is a fatal, progressive paralysis arising from the premature death of motor neurons. An understanding of the role of muscle in ALS has practical implications for treating the disease. Transgenic (Tg) mutant hSOD1^{G93A} mouse develops progressive loss of spinal motor neurons, muscle atrophy, and paralysis. Retraction of motor axons from synaptic connections to muscle is among the earliest presymptomatic event. Even if recent evidence suggests that mutant SOD1-mediated damage within muscle is not a significant contributor to non-cell-autonomous pathogenesis of ALS, loss of muscle proteins and differential changes in muscle proteome could act as signalling events inducing progressive damage both at the muscular and motorneuronal levels.

Objectives: The aim of the study is to detect possible biomarkers of primary events in skeletal muscles of the Tg hSOD1^{G93A} animal model and to translate the findings to the muscle of ALS patients. The identification of biological markers is critical for an early diagnosis of ALS in the hope that neuroprotective therapies may be more beneficial.

Methods: We examined the proteomic profile of gastrocnemius muscle from Tg hSOD1^{G93A} mice at presymptomatic (7 weeks) and symptomatic (14 weeks) stages of the disease and of control wild-type (NTg, 7 and 14 weeks) and after crush of the sciatic nerve by two dimensional difference in gel electrophoresis (2D-DIGE) and mass spectrometry. The same proteomic investigation was applied to vastus lateralis muscles from 8 sporadic ALS cases and one SOD1-mutated (L144F) patient. Healthy age-matched subjects were considered as controls.

Results: Differential analysis of muscles at the early presymptomatic age compared to NTg, revealed that 82 spots were significantly and differentially expressed in Tg hSOD1^{G93A} versus NTg mice. 24 of them were identified by mass spectrometry. At 14 weeks of age, quantitative differential analysis of Tg hSOD1^{G93A} vs. NTg generated 153 differentially expressed spots. 55 are common to the two different disease stages, and 16 of them were identified. Among identified proteins, 8 were differentially expressed at 7 weeks and these changes were absent 7 weeks later. All the proteins were mitochondrial. Only spots exclusively changed during disease progression or presenting differential behaviour from ageing and crush where taken into consideration. Proteome alteration in Tg mice represents a logical referral for analysis in ALS patients.

Conclusions: A further understanding of the role of muscle in ALS is essential and may have many practical implications. A comparative proteome analysis in ALS animal models and patients represents a step forwards to the definition of markers for early diagnosis and treatment of the disease.

C68 OXIDATIVE STRESS AND RELATED BIOMARKERS IN PATIENTS WITH SPORADIC ALS

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Background: Oxidative stress/injury is one of the key pathogenic processes in motoneuronal degeneration in ALS. A number of oxidative biomarkers such as DNA adducts, lipid peroxidation products, and protein oxidative adducts are found to be abnormal in body fluids (CSF, plasma and urine) in patients with ALS using high pressure liquid chromatography (HPLC) coupled with mass spectroscopy or electrochemical detection (ECD) methods, considered as the gold standard for measuring oxidative stress markers. We need simple and reliable methods to measure oxidative stress biomarkers for molecular epidemiological studies.

Objective: To investigate oxidative stress biomarkers using ELISA methods and other associated biomarkers in a cross section of 49 patients with sporadic ALS (sALS) compared to the available 46 healthy control subjects (spouses, siblings, and friends).

Methods: We measured urinary 8-oxodeoxyguanosine (8-oxodG), urinary15-F_{2t}-isoprostane (IsoP) levels, and plasma protein carbonyl by ELISA methods. In the same urine, 8-oxodG was measured by an HPLC/ECD method to correlate between the two methods, and in addition, paraoxonase 1 was measured by a well-established photo-spectroscopic method, and human cytokines using a kit for TNF-α, IL-1β, IL-2, MCP-1, IL-8, IFN-γ, IL-10, IL-6, IL-4, IP-10, IL-12p40, and MIP-1α run on a Luminex^{100}.

Results: The sALS patients were older than controls and a greater proportion were male. Urinary IsoP and urinary 8-oxodG concentrations were higher among cases than among controls, before adjustment for urine concentration (urinary creatinine). As expected, 8-oxodG increased with age ($r=0.25$, $p<0.02$). No relationship was found between urinary IsoP/creatinine and age. Urinary 8-oxodG ($p=0.018$) and urinary IsoP ($p=0.002$) levels both adjusted by urinary creatinine were significantly elevated in patients with sALS. These significant differences persisted after controlling for age and gender in logistic regression analyses. These two markers are markedly correlated with each other by the Spearman correlation ($r=0.70$; $p<0.0001$). Urinary 8-oxodG measured by the ELISA technique was significantly correlated with the measurement by HPLC/ECD in the same urine sample ($r=0.55$; $p<.0001$). Neither plasma protein carbonyl levels nor paraoxinase 1 showed significant differences between patients and controls. There were no significant changes in cytokines or chemokines in patients with ALS. However, IP-10 levels were correlated with 8-oxodG ($r=0.32$; $p=0.008$).

Conclusion: Our study confirmed that oxidative stress biomarkers as measured by the ELISA method were abnormally elevated in patients with sALS. To measure 8-oxodG, the ELISA method was found reliable against the gold standard method, HPLC/ECD. The ELISA method can be used in the future for molecular epidemiological studies requiring a large sample to determine the significance of increased oxidative stress biomarkers and epidemiological evidence of oxidative stress in sALS.

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C69 MR SPECTROSCOPY FINDINGS IN RECENT ONSET MOTOR NEURON DISEASE

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Background: Signs of upper *and* lower motor neuron lesion (UMN and LMN) are mandatory to diagnose amyotrophic lateral sclerosis (ALS), but the LMN lesion often dominates the clinical picture. It has not been settled whether the *primary* target of disease is the UMN, LMN, or both. Progressive muscular atrophy (PMA) is a LMN disorder, but at autopsy UMN involvement can be observed and PMA often evolves into ALS in due course. Primary lateral sclerosis (PLS) is a pure UMN disorder. MR spectroscopy (MRS) allows for assessment of corticomotoneuronal dysfunction through measurement of N-acetyl aspartate (NAA) concentration, and of glial activity through measurement of Myo-Innositol (MI). The ratio of NAA/MI was recently found to be a promising biomarker given its optimal sensitivity and specificity profile (1).

Objectives: To investigate longitudinally early involvement of the upper motor neuron (UMN) in motor neuron disease (MND) subtypes using proton MRS.

Methods: Patients with bulbar-onset ALS (n=12), limb-onset ALS (n=12), PMA (n=12), PLS (n=12) and healthy controls (n=12) were enrolled.
All patients had weakness for <1 year, except for the PLS group. Single-voxel spectroscopy (PRESS, TE=35, TR=2000) was performed in the primary motor cortex on a 3.0 Tesla scanner, using LC model for analysis. Metabolite concentrations in patient groups were compared to controls using a Mann-Whitney U test. Follow up visit was at 6 months.

Results: At baseline we observed a significant decrease in NAA ($p=0.000$) and a significant increase in MI

(p=0.024) in the PLS group. In limb-onset ALS the ratio of NAA/creatine (p=0.013) and of NAA/MI (p=0.024) was significantly decreased. In bulbar-onset ALS the ratio of NAA/creatine was significantly decreased (p=0.005). There was a trend towards increased levels of MI in both ALS groups. Metabolite concentrations in PMA were normal. Glutamate levels were equal in all groups. Finger/foot tapping speed (a feature of UMN involvement) was significantly decreased in PLS (p=0.000/p=0.000), in limb-onset ALS (p=0.009/p=0.019) and in bulbar-onset ALS (p=0.045/p=0.143), but not in PMA. Follow up data will be presented.

Discussion and conclusions: In the PLS group neuronal loss and active glial involvement was most prominent. In both ALS groups the decrease in NAA levels indicates early corticomotoneuron involvement. Furthermore, the trend towards an increase of MI in ALS suggests active glial involvement, also at an early stage of disease. We could not confirm that the ratio of NAA/MI is more sensitive and specific than NAA alone in ALS patients. The absence of relevant changes in NAA and MI in PMA and the normal speed of finger/foot tapping support the purely LMN origin of this disorder.

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C70 GENOME WIDE ASSOCIATION STUDY OF ALS IDENTIFIES TWO NEW SUSCEPTIBILITY GENES

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Background: Recent technological advances and the completion of the HapMap project have made it possible to screen the entire genome for association with ALS.

Objectives: To identify novel genetic risk factors for sporadic ALS by screening all variants in the human genome in an unbiased way.

Methods: We performed a genome-wide association study in 461 Dutch cases and 450 Dutch controls with the Illumina 300K SNP chips and replicated our findings in independent studies from the Netherlands, Belgium, Sweden and the USA.

Results: We identified 2 SNPs in 2 different genes that were significantly associated with ALS after correction for multiple testing. For one of the genes we could also demonstrate highly significant overexpression in ALS cases.

Discussion: One of the genes is involved in glutamate signalling and apoptosis and is therefore a very strong candidate for a susceptibility gene.

C71 GENETIC VARIANTS OF ELP3 ARE ASSOCIATED WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: ALS shows complex inheritance. In about 10% of cases there is a family history usually consistent with autosomal dominant inheritance and age-dependent penetrance. Mutations in the gene for cytosolic superoxide dismutase, *SOD1*, are detected in about 20% of familial and 3% of sporadic cases. Other evidence of a genetic contribution comes from non-*SOD1* linkage studies, and a twin study showing a heritability of between 0.38 and 0.85. Despite many candidate gene studies, the genes critical for the complex inheritance of the majority of ALS cases remain unknown.

Aims: To identify genes associated with sporadic ALS.

Methods: We examined samples from three geographically distinct populations: the UK, US and Belgium in a multi-stage design using the UK set as a discovery sample and the other two populations as validation sets. To prioritize markers for further study, the UK population was genotyped in DNA pools, with the highest priority markers re-genotyped in individuals. Markers confirmed as being associated in the UK population were then

re-genotyped in the Belgian and US populations. Replicated associations were tested by SNP genotyping in the region. Markers were selected by targeting to candidate genes and regions or using a gene-density targeting approach with the program MaGIC. Statistical analysis for the DNA pools was by metaregression of caseness against allele frequency incorporated in the program STATA and weighted by the inverse of the sampling and measurement variance for each pool. Individual genotypes were analyzed by chi-square test and a stratified analysis by Manel-Haenzsel chi-square test.

Results: There were 782 cases and 701 controls. In the UK population, 2,336 microsatellite markers were tested. In the DNA pooling stage, of the 12 highest ranked markers there were two pairs within 1 Mb of each other, one pair on chromosome 3, the other on chromosome 8. When typed in UK individuals, all were confirmed as showing association. When tested in the validation populations, the same alleles (6 and 10 of D8S1820, a 15 allele marker) were associated with a reduced risk of ALS in all three populations, being significantly under-represented in cases compared with controls (Mantel-Haenzsel chi-square test $p = 1.2 \times 10^{-7}$), with an estimated common odds ratio of 0.48, 95% confidence interval 0.36–0.64. Marker D8S1820 lies in intron 10 of the *ELP3* gene, a component of the RNA polymerase II complex. 54 tagging SNPs in *ELP3* were tested for association with ALS. No single SNP was associated with ALS in all three populations suggesting that the microsatellite itself is functional or that a functional SNP or haplotype is not tagged by the markers used.

Conclusion: We have shown that normally occurring, polymorphic variants within the *ELP3* gene are associated with a reduced risk of sporadic ALS.

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SESSION 9A INFLAMMATION

C72 MICROGLIA: FRIEND OR FOE IN ALS?

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The significance of microglial neuroinflammation in the pathogenesis of ALS, as well as other neurodegenerative diseases, remains unresolved. Numerous studies have portrayed microglia as potentially dangerous immune effector cells, yet mechanisms of how microglia would selectively kill motoneurons in ALS remain elusive and speculative. We have been studying microglial activation after acute motoneuron injury (facial nerve axotomy) in rats, and these studies clearly suggest a neuroprotective rather than a neurotoxic function for activated microglia, because microglial activation accompanies spontaneous and successful regeneration of motoneurons after axotomy. We therefore view microglia as fundamentally neuroprotective glia of the CNS.

Our histopathological studies of microglia in human brains from normally aged individuals, as well as from those with Alzheimer's disease, have provided evidence that microglial cells themselves are subject to degenerative changes. These were documented as morphological abnormalities involving deramifications, bulbous swellings, and perhaps most significantly, fragmentations of cytoplasmic processes, and they are collectively referred to as microglial dystrophy. Considering the observations of microglial dystrophy against the background of the perceived basic neuroprotective roles of these cells, we were inspired to hypothesize that degeneration of microglia contributes to the development of neurodegenerative disease due to a loss of loss of constitutive microglial neuroprotection (microglial dysfunction hypothesis).

The objective of our most recent work has been directed towards uncovering evidence for microglial degeneration in ALS. To this end, we performed a histopathological evaluation of microglial neuroinflammation in the $SOD1^{G93A}$ transgenic rat. Multiple levels of the CNS from spinal cord to cerebral cortex were studied during three stages of natural disease progression, including presymptomatic, early symptomatic (onset), and late symptomatic (end stage), using immuno- and lectin histochemical markers for microglia, such as OX-42, OX-6, and *Griffonia simplicifolia* isolectin B4. Our studies revealed abnormal aggregates of microglia forming in the spinal cord as early as the presymptomatic stage. During the symptomatic stages there was prominent formation of multinucleated giant cells through fusion of microglial cells in the spinal cord, brainstem, and red nucleus of the midbrain. Other brain regions, including substantia nigra, cranial nerve nuclei, hippocampus and cortex showed normal appearing microglia. In animals during end stage disease at 4–5 months of age virtually all microglia in the spinal cord grey matter showed extensive fragmentation of their cytoplasm (cytorrhexis), indicative of widespread microglial degeneration. These findings offer further support for the microglial dysfunction hypothesis by demonstrating that aberrant activation and degeneration of microglia occurs during the development of motor neuron disease.

C73 IMAGING IN MOUSE MODELS OF NEURODEGENERATIVE DISORDERS

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In recent years, imaging strategies employing reporter molecules have been developed to study biological processes as they occur in living animals or cell assays. One class of reporter genes that have been widely used for *in vivo* detection is fluorescent protein such as GFP. Another approach uses bioluminescence enzymes like the *firefly* luciferase (Fluc) that emit light when provided with the appropriate substrate. Luciferase catalyses the cleavage of the substrate luciferin in presence of oxygen and ATP, resulting in the emission of light with substantial fraction of light above 600 nm making it more suitable for *in vivo* imaging. The photons emitted by Fluc reporter activity pass the host tissue and are detectable at the surface using high-resolution and high-sensitivity CCD camera.

Although major clinical symptoms in amyotrophic lateral sclerosis (ALS) arise from neurodegeneration and death of motoneurons, recent studies suggest that glial cells play a role in the toxicity to motor neurons. Their precise role in onset and progression of the disease remains however unknown. To study the role of non-neuronal cells in the disease onset and progression, we developed mouse models for live imaging of glial activation. Glial fibrilary acidic protein (GFAP) is strongly up-regulated in ALS and its up-regulation has been associated with the disease progression. To generate a mouse model for live imaging of astrogliosis, we took advantage of reporter mice carrying the firefly luciferase gene under the transcriptional control of GFAP promoter (GFAP-luc, Caliper-Xenogen, CA) and crossed them with $SOD1^{G93A}$ mutant mice. Live imaging of astrocyte activation was performed weekly starting as early as 3–4 weeks of age till the end stage of the disease using a high resolution CCD camera for small animal optical imaging. Data collected by live imaging showed that photon emission/GFAP signals were detected very early in the pre-onset phase of disease in the lumbar spinal cord area. The signal first arose from small multiple areas of astrocyte activation which then converged into a larger signal around 80–100 days of age. Moreover the peak signals arising from the spinal cord at approx. 100 days correlated with the abrupt onset of sensori-motor deficits and paralysis.

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In conclusion, the transgenic reporter mice will provide unique tools for understanding disease pathology and longitudinal responses to drug testing. Such *in vivo* imaging technologies offer new avenues for rapid identification of drugs with potential clinical applications as they yield more predictive preclinical data that will facilitate understanding of disease processes and achieving safe and effective treatment.

C74 THE ADAPTIVE IMMUNE SYSTEM MEDIATES MICROGLIA RESPONSES TO INJURY IN AN ANIMAL MODEL OF ALS

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Background: Dominant mutations in superoxide dismutase (mSOD1) cause a familial form of amyotrophic lateral sclerosis (ALS), a progressive, paralytic disease characterized by loss of motoneurons. Previous studies from our laboratory demonstrated the presence of cells from the adaptive immune system in the spinal cords of ALS patients and in mice over-expressing mSOD1. We also demonstrated *in vitro* that the over-expression of mSOD1 in microglia causes these innate immune cells to be more activated and to induce more motoneuron death than wild-type (WT) microglia. More recently, we established that the innate immune system plays a pivotal role in determining the rate of disease progression in mSOD1 mice bred with PU.1$^{-/-}$ mice, mice that are unable to develop myeloid and lymphoid cells. WT bone marrow transplants (BMT) into mSOD1\PU.1$^{-\backslash-}$ mice repopulated the central and peripheral immune systems, slowed the loss of motoneurons, prolonged survival, and increased disease duration by 40 percent.

Objectives: Because this *in vivo* study modified both the peripheral and central immune systems, these results do not preclude the influence of the adaptive (T- and B-cells) immune system regulating the microglial response and its role in motoneuron injury. Thus, this current study was designed to determine the potential role of the adaptive immune system in mice overexpressing mSOD1, a transgenic model of familial ALS.

Methods and Results: We bred mSOD1 mice with RAG2$^{-/-}$ mice, mice that are unable to develop mature and functional T- and B-cells, and with RAG2$^{+\backslash-}$ mice. Although age of onset was not different, mSOD1\RAG2$^{-\backslash-}$ mice died earlier than SOD1\RAG2$^{+\backslash-}$. Following irradiation and mSOD1- or WT-derived BMT, survival was extended in mSOD1\RAG2$^{-\backslash-}$ mice back to the length of time observed with mSOD1\RAG2$^{+\backslash-}$ mice. Immunohistochemical analyses of spinal cord sections from end-stage mSOD1\RAG2$^{-\backslash-}$ mice demonstrated drastically reduced CD11b signal compared with that observed in mSOD1\RAG2$^{+\backslash-}$ mice or transplanted mSOD1\RAG2$^{-\backslash-}$ mice. BMT restored T-cells at sites of injury and increased CD11b expression in irradiated mSOD1\RAG2$^{-\backslash-}$ mice.

Discussion and conclusions: These data indicate that the state of microglial activation is influenced by the presence of T-cells, and possibly B-cells, in mSOD1 mice. In the absence of functional T- and B-cells, there is an enhanced toxicity, suggesting that the interaction of T- and/or B-cells with microglia mediates neuroprotection. The immunohistochemical data are also suggestive of neuroprotection mediated by the adaptive immune system. In agreement with the optic (1) or facial nerve (2) injury models, this study suggests that neuroprotection involves the communication between peripheral immune cells and central microglia and that T- and/or B-cells regulate the state of microglial activation. This study validates immunomodulatory therapies directed at maximizing neuroprotection in ALS.

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C75 ACTIVATION OF PERIPHERAL BLOOD CD16+ MONOCYTES IN ALS IS MEDIATED THROUGH TOLL-LIKE RECEPTORS AND NF*K*B-MEDIATED SIGNALLING

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Background: Chronic stimulation of innate immunity via Toll-like receptors (TLRs) has been associated with exacerbation of disease in the SOD1 mouse model. CD16+ monocytes isolated from ALS patients upregulate a characteristic subset of genes which include a number of genes that are also upregulated in lipopolysaccharide (LPS) stimulated macrophages in vitro.

Objective: To determine if CD16+ macrophages in ALS patients are activated via Toll-like receptor (TLR) mediated signalling.

Methods: Blood samples were obtained from patients with ALS and healthy controls after obtaining informed consent. White blood cells were purified and cultured in suspension in RPMI media plus 10% FBS in the presence of various TLR agonists including LPS (TLR4), lipoteichoic acid (TLR2), and poly I:C (TLR3). RNA was isolated and aliquots of the RNA preparations were analyzed by quantitative RT-PCR with primer pairs specific for the genes G1P3, GPR43, ORM1, CHI3L1, IL1RN and CD14. Results were normalized to β-actin, and / or time 0 RNA levels.

Results: ALS patient CD16+ monocytes co-ordinately upregulate RNA expression of genes associated with NFκB mediated transcription and type I interferons. Attempts to induce similar gene transcription patterns in normal monocytes with TNFα, conditioned media from

ALS patient mononuclear cells, or plasma from patients with ALS were unsuccessful. In contrast, exposure of mononuclear cells from healthy individuals to 10 ng/ml of LPS but not other TLR agonists induced approximately 10 fold induction of transcription of 6 indicator genes in 3 hours as seen in *unstimulated* ALS patient mononuclear cells. Stimulation of the indicator genes was inhibited by the broad spectrum NFκB inhibitor pyrrolidine dithiocarbamate (PDTC) in both ALS patient and control LPS-stimulated mononuclear cells. Variable inhibition was seen with resveratrol, an inhibitor of Myd88-independent TLR (primarily TLR3 and TLR4) signalling.

Conclusions: Peripheral blood macrophages from individuals with sporadic ALS are chronically stimulated via the Toll Like receptor / NFκB mediated pathways to an activated pro-inflammatory state. This chronic stimulation of the innate immune response has the potential to accelerate the disease course. Conversely, treatments that restore macrophages to a quiescent state have the potential to ameliorate the disease.

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SESSION 9B CLINICAL TRIALS AND TRIAL DESIGN

C76 RESULTS AND IMPLICATIONS OF A PHASE III RANDOMIZED CONTROLLED TRIAL OF MINOCYCLINE

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Background: Considerable laboratory evidence suggests that minocycline possesses anti-apoptotic and anti-inflammatory activity, and may act as a neuroprotective agent. Four published reports showed that minocycline extended survival in the SOD-1 rodent model. The potential neuroprotective effects of minocycline have been examined in models of other neurological conditions and numerous trials are planned to assess whether minocycline may slow the course of human neurodegeneration.

Objectives: To examine the efficacy and safety of minocycline in a phase III ALS trial.

Methods: We conducted a randomized placebo-controlled trial of minocycline at 31 centers in the United States. After a 4 month lead-in phase, 412 patients were randomized to either minocycline in escalating doses up to 400 mg/day, or placebo, for 9 months. Patients underwent monthly evaluations during their 13 months of participation. The primary outcome measure was the difference in rate of change in the revised ALS Functional Rating Scale (ALSFRS-R) between groups. Secondary outcome measures were forced vital capacity (FVC), manual muscle testing (MMT), quality of life (QOL), survival, and safety. Analyses of efficacy outcomes were performed using a linear mixed-effects model. Adverse events were assessed with Fisher's exact tests. The study had 80% power (alpha =0.05) to detect an 18% difference in the ALSFRS-R change in slope.

Results: The ALSFRS-R deteriorated at a 25% faster rate ($p=0.005$) in the minocycline group than in the placebo group. FVC (15%; $p=0.11$) and MMT (14%; $p=0.11$) tended to deteriorate more rapidly in the treated group. There were no differences in survival, QOL, or serious adverse events, but gastrointestinal ($p<0.001$) and neurological ($p=0.02$) non-serious adverse events were more common in the minocycline group.

Conclusions: High-dose minocycline has a harmful effect on ALS as measured by the ALS Functional Rating Scale. While the differences were small, and no changes were seen in survival time or quality of life, these results have implications for other trials of minocycline in neurological diseases, and for the current approach to screening potential neuroprotective agents in ALS. The presentation will include additional post hoc analyses that examine potential causes of the worsening due to minocycline, including dosage, adverse events, and interaction with riluzole.

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C77 COMBINATION DRUG SELECTION TRIAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Combining agents that affect different mechanisms of neurodegeneration may be necessary to reach meaningful outcomes in ALS trials. The combinations of minocycline/creatine and celecoxib/creatine have additive effects in the SOD-1 model. Novel phase II trial designs are needed to efficiently screen the growing number of potential neuroprotective agents.

Objective: To compare the neuroprotective capacity of two drug combinations in a phase II selection trial using group sequential design and comparison to a natural history control group for a futility analysis.

Methods: We conducted a randomized, double-blind selection trial of combination therapy, with a planned enrollment of up to 120 patients in sequential pools of 60. Participants were randomized to either minocycline (100 mg BID)/creatine (10g BID) or celecoxib (400 mg BID)/creatine (10 g BID), and were evaluated monthly for six months. The primary objective was treatment selection based on which drug combination appeared to best slow deterioration in the ALSFRS-R. After the first pool completed 6 months, the trial could be stopped if the difference in ALSFRS-R scores between the two arms was adequately large (defined as 0.75 times the standard error of the average score). If neither combination could be selected after one pool, an additional 60 patients would be randomized. A secondary objective was evaluation of futility. At trial conclusion, the decline in ALSFRS-R in both treatment arms was compared separately to the mean in a historical control group at a 0.05 significance level. The null hypothesis stated that the selected treatment confers at least a 25% reduction in the ALSFRS-R compared to the controls.

Results: Of the 60 patients enrolled in pool I, 58 completed all 6 months and 2 died; there were no dropouts. The mean declines in ALSFRS-R were 6.47 (SD=9.14) in the minocycline/creatine arm, 5.27 (5.54) in the celecoxib/creatine arm, and 5.82 (6.77) in the historical control group. The pooled sample standard deviation of the change in ALSFRS-R was 7.556; the numerical threshold for the selection criterion was 1.035 ($0.75 \times 7.556/\sqrt{30}$). The absolute difference between the two sample means was 1.20, exceeding the threshold in the selection stopping criterion. Next, we failed to reject the null hypothesis of superiority in the futility analysis.

Conclusions: The selection criterion was met following pool I. We were able to close the trial early, selecting the celecoxib/creatine combination, which had a smaller mean decline than the minocycline/creatine arm, and was non-futile in comparison to the historical controls. These data can now be used to design a phase III trial of combination therapy in ALS. This phase II construct was efficient, leading to treatment selection after just 60 patients, and could be used in larger phase II selection trials assessing greater numbers of agents.

C78 TRO19622 IS WELL TOLERATED AND TARGET PLASMA CONCENTRATIONS ARE OBTAINED AT DOSES 250/500 MG ONCE A DAY IN A ONE MONTH ALS PHASE 1B, ADD-ON TO RILUZOLE, CLINICAL STUDY

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Background: TRO19622, a low molecular weight cholesterol-like molecule (cholest-4-en-3-one,oxime), is a new chemical entity that enhances motor neuron survival and nerve regeneration in multiple paradigms *in vitro* and *in vivo*.

Method: A double-blind, randomized, repeated-dose, placebo-controlled clinical study was conducted in 18 males and 18 post-menopausal females (mean age 59 years) with a probable/definite diagnosis of amyotrophic lateral sclerosis. The study explored the safety, tolerability and trough plasma levels of 3 doses of TRO19622 given once daily for one month as add-on to riluzole (RLZ) 50 mg BID (morning and evening). Three doses of TRO19622, 125, 250 and 500 mg QD *per os* just before the noon meal were administered for one month in 3 groups of 12 patients (9 on TRO19622+RLZ, 3 on placebo+RLZ). ECGs were performed weekly and centrally read by a cardiologist. Laboratory tests were performed at inclusion and on Days 15 and 30 and at a follow-up visit on Day 45. The ALS Functional Rating Scale (ALS FRS-R) at inclusion was high and comparable between dose groups with an overall mean ($\pm$SD) of 38.6 ($\pm$3.5) . Mean Slow Vital Capacity (% of predicted value) was comparable between dose groups with an overall mean of 97.7 ($\pm$21.9).

Results: All doses were well tolerated. Sixty-nine treatment emergent adverse events (TEAEs) were reported: 13 were considered possibly related to drug, 2 probably related, 21 unlikely related and 33 were considered unrelated to drug. The two probably related TEAEs occurred in the control group. Among the possibly related TEAEs, 1 occurred in the control group, 6 in the 125 mg group, 3 in the 250 mg group and 3 in the 500 mg group. These TEAEs were mild (n=55), moderate (13) or severe (2) in intensity. The most frequent TEAEs ($n \geq 4$ episodes) were asthenia (12 episodes, 9 on TRO19622), diarrhoea (6 episodes, 4 on TRO19622), constipation (3 episodes, 1 on TRO19622) and muscle spasms (4 episodes, 3 on TRO19622). The frequency, severity, duration and time to TEAEs did not increase with dose. One serious adverse event , a deglutition pneumonia, was reported in the 500 mg group and assessed as unrelated to treatment. No relevant changes in vital signs, ECG parameters, laboratory tests or physical examinations were observed at any time and in any dose group. As anticipated in a one month study, there was no change in ALSFRS-R and Slow Vital Capacity. Day 15 and Day 30 TRO19622 plasma trough

concentrations were similar suggesting that steady state had been reached. Median male and female trough concentrations were 512 and 742 ng/mL (125 mg group), 979 and 1685 ng/mL (250 mg) and 2965 and 3310 ng/mL (500 mg). The highest trough concentration, 5780 ng/mL was observed on day 15 at 500 mg. There was no statistically significant gender effect at all three TRO19622 doses ($p > 0.32$, Mann-Whitney) at day 15.

Conclusion: In conclusion TRO19622 up to 500 mg QD *per os* as add-on to RLZ 50 mg BID for one month was very well tolerated in ALS patients. Target plasma concentrations, based on *in vitro/in vivo* models, were obtained at the 250/500 mg doses. Higher trough TRO19622 plasma concentrations were observed in ALS patients vs healthy volunteers, either due to co-administration with food/RLZ or to some unknown factor specific to ALS patients.

C79 DOUBLE-BLIND, PLACEBO CONTROLLED SAFETY STUDY OF RITONAVIR AND HYDROXYUREA IN PATIENTS WITH ALS

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Background: To date riluzole is the only FDA drug approved to treat ALS. All other drugs tested to date either had no efficacy or caused more rapid progression compared with the placebo group. Hydroxyurea halts cell cycle transition at the G1/S phase via ribonucleotide reductase inhibition. Ritonavir reduces the levels of the inducible proteasome subunits LMP2 and LMP7, which are observed to be selectively up-regulated in ALS.

Objectives: To determine the safety of Ritonavir and Hydroxyurea in ALS patients in a phase I trial.

Methods: Based on initial efficacy studies in G93A SOD-1 mice, a safety trial was initiated at our ALS Center with a placebo control, given the known toxicity of these drugs. Patients were enrolled with definite, probable, or laboratory supported probable ALS according to El Escorial Revised Criteria with an FVC $>50\%$ and disease duration <5 years. Patients were randomized to low dose Ritonavir at 200mg BID, high dose Ritonavir at 400mg BID, Hydoxyurea at 1000mg QD or placebo. Patients were followed monthly for 6 months and given an option of open label treatment at the end of the study and followed monthly for an additional 6 months. ALSFRS-R was the primary outcome measure. FVC, MIF, and weight were measured monthly. At 3 month intervals manual muscle strength was measured by both a neurologist and physical therapist to assess inter-rater reliability.

Results: After enrolment of 24 patients, an interim safety analysis was performed. Patients in the high dose Ritonavir arm compared to placebo had a 5 fold decline in FVC ($p < 0.0001$), a 19 fold decline in weight ($p < 0.0001$), a 3 fold decline in MMT ($p < 0.0001$), and the decline in ALSFRS was unchanged. The other arms of the study did not show significant differences in the above measures compared to placebo at the time of the interim safety analysis. Based on these findings the high dose Ritonavir arm was eliminated from the study. Final results will be presented at the meeting, as the last patient will finish the study in July, 2007.

Discussion and conclusions: Performing a safety study testing 2 potential efficacious drugs for ALS with a placebo control allowed us to identify a toxic drug early in the study and to eliminate the high-dose arm based on an interim safety analysis. This may serve as a model for other phase 1 studies to help avoid drugs proceeding to phase II trials that are toxic to ALS patients.

C80 DESIGN OF PHASE II ALS CLINICAL TRIALS

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Background: In the past decade, there have been several phase II and III clinical trials in ALS. The pipeline of new therapies for testing is large and the field requires more efficient methods to test new agents. Proposals have included ''futility'' (1) and ''lead in'' (2) designs, multi-drug screening trials, combining phases of therapy, and using group sequential methods (3). In a futility design the null hypothesis is that the treatment is better than placebo, if it is rejected the new treatment is not tested further. In a lead in design all patients are observed for, say, three months before being randomized between treatment and placebo. Multi drug screening trials compare multiple drugs and test the best drug further and group sequential methods allow trials to stop early for efficacy or futility.

Objectives: We present a discussion and critique of the major proposals in the design of clinical trials in ALS.

Methods: We compare the relative efficiency of designs using the ratio of the sample sizes required to detect the same difference. Efficiency is calculated analytically or by simulation.

Results: The use of ''futility designs'', that are designed to select drugs for further testing, will too often lead to the testing of ineffective drugs. This occurs because the alternative hypothesis for these designs is that the drug is worse than the placebo. If the drug is no different than the placebo futility may not be rejected and the drug tested further. The use of a ''lead in'' design doesn't reduce sample size without strong assumptions about linearity and lack of a placebo effect. Using data from previous clinical trials we found evidence of nonlinearity and we believe that a placebo effect is likely. We show that using multi-drug phase II trials to select drugs for further study could

halve the time required to find an effective treatment. The advantage of studies that combine phase II and III is that they have the potential of saving years in a publicly funded drug development program. We also describe the advantages and disadvantages of using group sequential methods to stop trials early. We discuss the pros and cons of using survival as a measure of efficacy as compared to functional status or strength.

Conclusions: The standard clinical trial design without a lead in is best for Phase II and Phase III clinical trials. This design could be preceded by a multi-drug screening study to identify which drugs to study first. Sequential trials have a role in the ALS drug development.

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C81 USE OF ACCELEROMETERS TO MEASURE DISEASE PROGRESSION IN ALS

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Background: Several indices of disease progression have been used as outcome measures in ALS trials including: survival, isometric muscle testing, grip dynamometry, manual muscle testing, timed motor activities, pulmonary functions, and functional rating scales. None of these provide objective quantitative data on motor performance in the home setting.

Objective: To determine if limb accelerometers will provide quantitative assessment of disease progression in ALS that could be used in the home as an outcome measure in future drug trials.

Methods: We studied 34 ALS patients and 10 normal controls longitudinally and quantified the number and intensity of all physical movements of 4 limbs over a 24 hour period using Actical accelerometers affixed to each limb. All spontaneous movements were recorded in 1 minute epochs and uploaded to the computer for analysis. Each participant recorded their activity during 15 min epochs using the Bouchard scaling method.

Results: Total activity counts from all limbs declined over time in ALS subjects. The total activity of healthy controls fluctuated depending on their participation in recreational activity. Activity counts in each limb were compared to muscle mass measured by DXA scanning, manual muscle testing, and self-rating of power. Activity counts were apportioned into 9 levels of Bouchard activity with increasing caloric expenditure based on the intensity of movements. As strength declined, the distribution of activity shifted to lower intensities in a monotonic fashion. Distributing quantitative activity counts in this manner permitted correlation with caloric equivalency of movements and with the subjects' self-ratings.

Conclusions: The use of accelerometers to measure disease progression has the advantages of providing quantitative information regarding the number of movements and their intensity. The devices are portable, light weight, water resistant, and can be used in the home. These attributes offer the possibility that they could be used as a primary outcome measure of disease progression in clinical drug trials. Moreover, their use may permit many future clinical trials to be conducted in the home with minimal travel to the medical centre, thereby decreasing dropouts.

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SESSION 10A CELL BIOLOGY AND PATHOLOGY

C82 AUTOPHAGIC CLEARANCE OF AGGREGATE-PRONE PROTEINS: THERAPEUTIC IMPLICATIONS

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Intracellular protein misfolding/aggregation are features of many late-onset neurodegenerative diseases, called proteinopathies. These include Alzheimer's disease, Parkinson's disease, tauopathies, and polyglutamine expansion diseases (like Huntington's disease (HD) and various spinocerebellar ataxias (SCAs), like SCA3). Currently, there are no effective strategies to slow or prevent the neurodegeneration resulting from these diseases in humans. The mutations causing many proteinopathies (e.g. polyglutamine diseases and tauopathies) confer novel toxic functions on the specific protein, and disease severity frequently correlates with the expression levels of the protein. Thus, the factors regulating the synthesis and clearance of these aggregate-prone proteins are putative therapeutic targets. The proteasome and autophagy-lysosomal pathways are the major routes for mutant huntingtin fragment clearance. While the narrow proteasome barrel precludes entry of oligomers/aggregates of mutant huntingtin (or other aggregate-prone intracellular proteins), such substrates can be degraded by macroautophagy (which I will call autophagy). We showed that the autophagy inducer rapamycin reduced the levels of soluble and aggregated huntingtin and attenuated its toxicity in cells, and in transgenic *Drosophila* and mouse models. Recently, we extended the range of intracellular proteinopathy substrates that are cleared by autophagy to a wide range of other targets, including proteins mutated in certain spinocerebellar ataxias, forms of alpha-synuclein mutated in familial forms of Parkinson's disease, and tau mutants that cause fronto-temporal dementia/tauopathy. I will consider the therapeutic potential of autophagy upregulation for various proteinopathies, and describe how this strategy may act both by removing the primary toxin (the misfolded/aggregate-prone protein) and by reducing susceptibility to apoptotic insults. I will also describe how we have identified novel mTOR-independent pathways which regulate autophagy and compounds that induce additive effects along with rapamycin.

C83 ENDOPLASMIC RETICULUM STRESS AND INDUCTION OF THE UNFOLDED PROTEIN RESPONSE IN HUMAN SPORADIC ALS PATIENTS

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Background: Sporadic and familial ALS have an identical clinical presentation, suggesting a common pathological mechanism. Cytoplasmic inclusions immuno-reactive for SOD1 are seen in human spinal cords in both forms of the disease. We showed recently that induction of the full unfolded protein response (UPR) including endoplasmic reticulum (ER) stress sensor kinases, chaperones and apoptotic mediators, occurs in transgenic $SOD1^{G93A}$ mice at symptom onset and disease endstage (1).

Objective: Although animal models expressing human mutant SOD1 proteins have been an invaluable disease model, SOD1-linked FALS represents only a small proportion of all ALS patients. Hence, we wanted to determine if UPR induction also occurs in human patient spinal cords, and whether ER stress is associated with sporadic disease or not.

Methods: Human patient lumbar spinal cord extracts were examined by Western blotting and immunohistochemistry for the up-regulation of UPR markers in comparison to normal controls.

Results: UPR sensors and chaperones were all up-regulated in human ALS patient spinal cords, implying that ER stress plays a role in the forms of disease unrelated to mutant SOD1. Furthermore, the ER chaperone PDI formed large cytoplasmic inclusions in the remaining patient motor neurons. Previously we showed that PDI binds to SOD1 expressed in NSC-34 cells and co-localises with mutant SOD1 inclusions. In addition, pharmacological inhibition of PDI increased the number of inclusions whereas overexpression of PDI decreased aggregate formation. Our combined results suggest that PDI may play a neuroprotective role in sporadic and familial ALS.

Conclusion: Our findings implicate ER-stress in the pathology of the more common, sporadic forms of disease as well as the SOD1-linked familial forms. In addition, PDI may be a novel therapeutic target for ALS.

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C84 PROTEASOMAL INVOLVEMENT IN STRESS RESPONSES IN ALS AND AGING

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Background: Altered protein solubility and aggregation in familial and sporadic ALS implicates failure of pathways for detecting and catabolizing misfolded proteins. Our previous studies showed early reduction of chaperoning (1) and proteasome-mediated proteolytic activities (2) in lumbar spinal cord of $SOD1^{G93A}$ transgenic mice, tissue particularly vulnerable to disease.

Objectives: To identify alterations in proteasomal structure and composition underlying decreased chymotrypsin-like activity.

Methods: Homogenates of lumbar spinal cord from $SOD1^{G93A}$ transgenic mice and non-transgenic littermates, as well as spinal cord of ALS patients and age-matched controls were subjected to 2-D blue native-SDS/PAGE and I-D non-denaturing PAGE followed by analysis of proteasomal subunit expression and incorporation into 20S/26S proteasomes by Western blotting. Levels of reverse-transcribed mRNA were assessed by real time PCR.

Results: Decrease in chymotrypsin-like activity was accompanied by decreased detection of the catalytic subunit, beta 5, and a structural subunit, beta 3, in 20S/26S proteasomes and accumulation of insoluble ubiquitinated substrates and mutant SOD1. These changes were not accounted for by reduced transcription/mRNA stability or by upregulation of immunoproteasome subunits (manuscript submitted). Current studies are addressing whether the apparent reduction in beta subunits results from post-translational modification masking immunodetection or substitution of novel stress-induced subunits. Proteasomes are being purified and analyzed by 2-D gel electrophoresis and subunits are identified by mass spectrometry. Post-translational modifications are investigated as previously described (3).

Discussion: Similar reduction in proteasomal catalytic activity and levels of beta 5 relative to 20S alpha subunits have been reported in spinal cord of aging rats (4) and in senescent fibroblasts (5). Beta 5 appears to be key regulator of proteasome assembly, as its expression increases biogenesis of proteasomes, delays senescent phenotype, and increases resistance to oxidative stress[5]. A better understanding of how proteasomes are involved in stress responses is required, given the central role of protein misfolding in ALS and other neurological disorders. *Funded by CIHR, Neuromuscular Research Partnership and ALSA*

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C85 ALTERATIONS OF UBIQUITIN PROTEASOME PATHWAY IN SPINAL MOTOR NEURONS OF SOD1G93A MICE

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Background: Accumulation of ubiquitinated proteins is a pathological hallmark of familial and sporadic Amyotrophic Lateral Sclerosis (ALS) and occurs in the spinal cord of transgenic mice carrying human mutant SOD1, a disease model. Alterations in the ubiquitin proteasome pathway (UPP) might influence the aggregate formation, however, it is still debated whether impairment of the UPP occurs in the motor neurons and/or in glial cells and if it is related to protein aggregate accumulation.

Objectives: To analyze: 1) the expression of different subunits of constitutive proteasome (CP) and immunoproteasome (IP) in spinal cord of SOD1G93A mice during the disease progression and 2) the activity of UPP *in vivo* at the cellular level, in SOD1G93A (G93A) carrying a constitutive and ubiquitous expression of Ub^{G76V}-GFP reporter for proteasomal degradation.

Methods: We used G93A mice at various stages of disease progression and non transgenic (Ntg) littermates. The mRNA for each subunit was analysed by Real Time-PCR. The cellular distribution of each subunit was examined by immunohisto-fluorescence and confocal microscopy. To examine the UPP *in vivo*, SOD1G93A mice were cross-bred with UPP reporter mice with constitutive and ubiquitous expression of Ub^{G76V}-GFP reporter for proteasomal degradation. Immunohisto-fluorescence of GFP was examined in combination with phosphorylated neurofilaments (SMI31), ubiquitin (signs of degeneration) and human SOD1 in double transgenic mice (UPP/SOD1G93A) at different stages of the disease.

Results: Compared to Ntg mice, a) presymptomatic G93A showed significant reduction of mRNA for CP 19S and IP 11S; b) symptomatic G93A showed decrease of 19S and 20S alfa CP mRNA and increased LMP7 and MECL1 IP mRNA; c) end-stage G93A showed decreased mRNA of all CP subunits while the mRNA of IP subunit LMP7 remained remarkably increased. Immunostaining of CP subunits decreased in motorneurons of symptomatic mice, but not in reactive glial cells that were also remarkably labelled with IP subunits. Immunohistochemistry on symptomatic and end-stage UPP/SOD1G93A mice showed in brainstem and spinal cord

regions few neurons, including motor neurons, that were clearly GFP positive. In ventral spinal cord of symptomatic mice about one third of GFP+ neurons contained somatic phosphorylated neurofilaments, while two thirds of GFP+ cells contained ubiquitin. GFP+ glial cells were never found. No changes in GFP mRNA were found in the lumbar spinal cord of symptomatic mice.

Conclusions: An early decrease of CP19S and IP11S transcript levels in ventral spinal cord may be the first signs of UPP change in G93A mice. The progressive decrease of CP subunits observed at the later stages may contribute to accumulation of protein aggregates found in neurons and neurites. This parallels the apparent reduced UPP function in GFP positive motor neurons in double GFP/SOD1G93A mice. *This work was supported by Telethon, Italy.*

C86 THE PROAPOPTOTIC PROTEIN BNIP3 PLAYS A ROLE IN MUTANT SOD1-INDUCED MOTOR NEURON DEATH IN ALS

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Oxidative stress, mitochondrial dysfunction and morphologically necrotic-like motor neuron death are major features of mutant SOD1-induced motor neuron death. To understand the molecular mechanisms, we focused on the mitochondrial protein BNIP3. BNIP3 is a proapoptotic Bcl-2/E1B-19kD-interacting protein. Previously we showed that oxidative stress provided a redox signal to activate hypoxia-inducible factor 1α (HIF-1α), the primary, if not the only, transcriptional factor for BNIP3 (1). Expression of BNIP3 caused a caspase-independent form of neuronal cell death *in vitro* and *in vivo* (2). We have recently tested the hypothesis that BNIP3 is involved in motor neuron death in ALS. Here we show that BNIP3 was induced to express 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 and in the G93A and the G37R mice before the onset of disease. Levels of BNIP3 expression increased with disease progression as evidenced by immunohistochemistry, Western blotting and RT-PCR analyses. The expressed BNIP3 was found to be primarily localized in 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. To determine whether the expressed BNIP3 was functional, mitochondria isolated from spinal cord tissue of the G93A mice were incubated on ice with freshly prepared 0.1 M Na_2CO_3 (pH 11.5) for 30 min, separated into membrane and S100 fractions and then Western-blotted using a BNIP3 antibody. BNIP3 was detected in the mitochondrial membranes after the alkaline extract, 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 nearly 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 μm in inner diameter) axons by the lentiviral BNIP3 shRNA vector. 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, and appears to be a new target for neuroprotective strategies in ALS.

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C87 A CASPASE-3 CLEAVED FRAGMENT OF EAAT2 IS SUMOYLATED AND TARGETED TO PML NUCLEAR BODIES IN MUTANT SOD1 LINKED ALS

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Background: EAAT2 is a high affinity, Na^+ dependent glutamate transporter of glial origin that is essential for the clearance of synaptic glutamate and prevention of excitotoxicity. During the course of human amyotrophic lateral sclerosis (ALS) and in the transgenic mutant SOD1 mouse model of the disease, expression and activity of EAAT2 is remarkably reduced. We previously reported that mutant SOD1 proteins exposed to oxidative stress inhibit EAAT2 *in vitro* in a cell model system and the impairment was largely brought about by Caspase-3 cleavage at a single defined locus, giving rise to two species that we termed truncated EAAT2 (Tr-EAAT2) and C-terminus of EAAT2 (CTE).

Objective: The objective of this study is to determine whether EAAT2 undergoes proteolytical processing mediated by caspase-3 *in vivo* in ALS.

Methods: To pursue this objective we analyzed spinal cord homogenates from transgenic mutant SOD1 mice as well as homogenates from spinal cord specimens obtained post-mortem from ALS patients.

Results: Analysis of spinal cords taken from mutant G93A-SOD1 mice reveals proteolytic cleavage of EAAT2 mediated by caspase-3. However, the proteolytic fragment corresponding to CTE was found to be of a higher molecular weight than expected due to conjugation with

SUMO-1. We found that the Sumoylated EAAT2 fragment accumulates in spinal cords of these mice as early as the pre-symptomatic stage of disease (at 70 days of age) and not in other CNS areas unaffected by the disease. The formation of this fragment is specific to the ALS mice as it does not occur in the R6/2 mouse model for Huntington's disease. Furthermore, we provide evidence using an astroglial cell line, primary culture of astrocytes and tissue samples from G93A-SOD1 mice that the novel species is targeted to promyelocytic leukemia nuclear bodies (PML-NBs).

Discussion: Taken together, our observations that a proteolytic fragment of EAAT2 is sumoylated, accumulates during the course of ALS and localizes in PML-NBs argue that it may play a role in the pathogenesis of the disease, an observation that is consistent with other studies implicating protein sumoylation in other neurodegenerative disorders. As one of the proposed roles of PML-NBs is regulation of gene transcription we suggest a possible novel mechanism for the role of the glutamate transporter EAAT2 in the pathology of ALS

C88 DEVELOPMENT AND CHARACTERIZATION OF A SOD1 ZEBRAFISH MODEL OF ALS

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Background: Although linkage analysis has been established with many different loci, only 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. Invertebrate models more amenable to genetic screens such as *C. elegans* and *Drosophila* have not mimicked the disease. Thus, additional animal models of ALS would benefit the field.

Objective: Our goal is to generate another vertebrate model of SOD1 FALS that is amenable to genetic analysis. Zebrafish offer a number of advantages as a system for modelling neuromuscular diseases including a well-characterized neuromuscular system, the ease of creating genetic mosaics, and the ability to perform mutant and suppressor screens.

Methods: We developed a transgenic zebrafish model of ALS carrying mutant or wild-type zebrafish *sod1* gene. The zebrafish *sod1* gene was identified by homology search with mouse *Sod* cDNA. A zebrafish BAC containing the genomic region of *sod1* was used to clone a 20 kb fragment containing the *sod1* gene and flanking sequences. This construct was injected into embryos at the early 1-cell stage. The injected fish (F0) were grown to adulthood and out crossed to wild-type fish. Founder (F1) fish were identified from these matings and the lines expanded.

Results: We currently have multiple lines of zebrafish carrying G93A, G85R and wild-type *sod1*. Copy number analysis by QPCR indicates that the copy number in the transgenics ranges from 4–100 copies, which could potentially be useful in studying dosage effects. We do not see axon defects or developmental defects in any of these fish at early stages of development. The highest copy transgenic G93A fish line showed symptoms of abnormal swimming as early as 4 weeks of age and showed disruption of the neuromuscular junction. These preliminary studies indicate that this zebrafish model mimics key aspects of ALS seen in humans and mice. Further detailed analysis of these mutant lines is ongoing.

Conclusions: We have successfully generated transgenic zebrafish carrying mutant forms of *sod1* and have observed phenotypes including abnormal swimming and denervated muscle. We also show that these fish, like mouse models and human patients, have no early developmental defects either in body development or motor axon outgrowth. We believe that the power of zebrafish lies in the ability to easily generate genetic mosaics to test cell-autonomy, the power of genetics to identify disease modifiers, and the potential to use this model in drug screens.

C89 THE ZEBRAFISH AS A NOVEL MODEL TO STUDY THE PATHOGENESIS OF ALS

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Background: The development of small animal models is of major interest to unravel the etiology, pathogenesis and treatment of neurodegenerative diseases. One such neurodegenerative disease is amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder characterized by the selective loss of motor neurons. Mutations in superoxide dismutase (SOD1) have been identified in a subset of patients with familial ALS (FALS). Furthermore sporadic ALS (SALS) is known to be a disease with complex genetics.

Objective: We have investigated the zebrafish *Danio rerio* as a potential model to study the pathogenesis of ALS by

evaluating the influence of mutant SOD1 on axonal outgrowth in the zebrafish embryo. A genomic association study using 2,336 microsatellite markers in three different populations was performed to find modifying genes in SALS, and the effect of *in vivo* functional knock down in the zebrafish of an identified gene on motor axonal outgrowth was determined.

Methods: MRNA of mutant and wild-type SOD1 was injected in embryos and motor axonal outgrowth was evaluated at 30 hours post fertilization. Abnormal branching was scored and total length of motor neuron axons was measured. To examine the potential of this approach for genes implicated in SALS we used a morpholino knock down technique in zebrafish embryos and determined whether axonal outgrowth in motor neurons was affected.

Results: Overexpression of mutant human SOD1 in zebrafish embryos resulted in aberrant motor neurons and was specific, dose-dependent and found for all three mutations studied. We investigated the role of a known modifier in the disease: vascular endothelial growth factor (VEGF). Lowering VEGF induced a more severe phenotype while upregulating VEGF rescued the mutant SOD1 axonopathy.
In three populations totaling 781 cases and 702 controls, the same common genetic variants within Elongator Protein 3 (ELP3) are associated with protection from SALS. *In vivo* knock down of ELP3 in the zebrafish induced motor axonal outgrowth defects.

Discussion and conclusions: Overexpression of mutant SOD1 induces a motor axonopathy in the zebrafish embryo. Moreover using this newly established model we underscore the potential of VEGF for the treatment of ALS. This novel model will permit large scale genetic and chemical screening to facilitate the identification of new therapeutic targets in motor neuron disease. It represents the first neuron specific zebrafish model for neurodegenerative diseases.
Knock down of ELP3, a gene associated with protection from SALS, clearly influences motor axonal outgrowth which implies its importance in motor neuron biology. This establishes the zebrafish embryo as a tool to determine the role of genes identified in genome wide association or candidate gene studies in SALS.

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SESSION 10B METABOLISM, NUTRITION AND RESPIRATORY FUNCTION

C90 HYPERMETABOLIC STATES IN ALS

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Despite the traditional view of ALS as a pure motor neuron (MN) disease, growing evidence suggests that the disease is, in fact, a multisystem disorder with additional extramotor neurological manifestations. Beyond the nervous system, intriguing metabolic alterations have also been observed in asssociation with the course of the disease. In particular, recent studies revealed that two thirds of ALS patients present with a stable hypermetabolism that correlates with survival.

The cause of the hypermetabolism in ALS is still unknown. Very early, transgenic mice with mutated SOD1 genes present with reduced adiposity and increased rates of energy expenditure, which reveals the presence of a metabolic deficit before any apparent sign of motor impairment. The burden of these metabolic alterations exerts some influence on the neurodegenerative process, since increasing the lipid content of the diet offers neuroprotection and extends survival (1). We have also shown that the gastroinstestinal absorption of lipids as well as the peripheral clearance of triglyceride-rich lipoproteins are markedly increased in the mutant SOD1 mice, which strongly suggests an increase in the consumption of lipids by muscles, a situation that could account for the protective effect of the high fat regimen in these animals (2). Our recent studies demonstrates that hyperlipidemia is a typical feature of ALS patients and, most importantly, that bearing an abnormally elevated LDL/HDL ratio significantly increased survival by more than 12 months (3).

An early hallmark of ALS is the destruction of the neuromuscular junction (NMJ), an event that might suggest that the anatomical target of MNs contributes to in the disease initiation. To test whether increased energy expenditure in skeletal muscle is sufficient to initiate denervation, we analyzed transgenic mice overexpressing uncoupling protein 1 (UCP1) under the control of the muscle specific creatine kinase (MCK) promoter. We showed that these mice present progressive denervation (as shown by electromyographic recording), loss of functional NMJ, loss of MN and muscle atrophy.

These results show that an increased energy demand in the muscle fibre is sufficient to initiate major disorders in the motor unit. The metabolic disorder associated with ALS may represent a physio-pathological mechanism whose importance in disease initiation and progression has been largely underestimated.

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C91 ALS PATIENTS HAVE A SIGNIFICANT HYPERACTIVITY AT ALL AGES: RESULTS OF A PROSPECTIVE STUDY

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Background: ALS has been frequently associated with heavy work and sport practice in the literature, but data largely remain in dispute. Studies of sub-groups such as professional soccer players, marathonians or military veterans suggest highly significant Odd Ratios as high as 20 in favour of an increased risk to develop ALS. We hypothesised that discrepancies between former studies could be due to the variety of activities making it unlikely, for example, for a mason to have time to practice sport and for a housewife with 4 children to have other activities. However, none of the previous published works have taken into account all the possible conditions leading to increased daily activity.

Objectives: To describe the daily energy expenditure (DEE) in ALS patients and to compare data with those from controls.

Methods: A questionnaire, validated by the WHO and FAO, was proposed to ALS patients and controls. This questionnaire collected daily data corresponding to the various activities of a person during the day. Each activity was associated to a number of Calories needed per hour. For example sleeping corresponds to 60Kcal/h, watching TV to 90Kcal/h and gardening to 170Kcal/h. Patients were questioned on all their activities for each decade from 20 years old. The control group was composed of subjects with other chronic neurological disorders (Parkinson's disease and syndromes, axonal neuropathy, multiple sclerosis etc.) excluding dementia and myopathies, hospitalized in our Neurology Department.

Results: There were 51 ALS and 40 controls in this interim analysis. The complete study aims to include 215 patients in each group. Age and gender were matched between ALS and controls. DEE between ALS and

controls were 2736 vs. 2317 (from 20 to 30 years old, p=0.00001), 2698 vs. 2338 (from 30 to 40, p=0.0001), 2675 vs. 2429 (from 40 to 50, p=0.02) and 2583 vs. 2242 (from 50 to 60, p=0.006). Data were also significant by gender, both for men and women. When analysing the items responsible for this increased activity, only those concerning heavy work were significantly higher in ALS. Sport practice did not show any increase between ALS and the control group.

Discussion and conclusions: DEE of ALS patients is significantly higher than controls with other chronic neurological disorders. This increase of DEE was noted both in men and women and could mainly be attributed to heavy daily work but not to sport practice. Heavy work has already been suggested as a risk factor in ALS but not confirmed by other authors. More than the heaviness of a given work, it appears that regular and intense daily activity is a more frequent way of life in ALS. It is not possible, at this stage, to determine to the exact relationship between ALS onset and such activities. However, we, and others, have recently shown that hypoxia may play a role in ALS etiopathogenesis. Such an increased DEE in the patients may be liable to aggravate the relative hypoperfusion or hypo-oxygenation of the motor neurons and, subsequently, could lead to neurodegeneration.

C92 EFFECTS OF TUBE-FEEDING ON QUALITY OF LIFE AND SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS (A COHORT STUDY IN 383 PATIENTS)

CLAVELOU P[1], OUCHCHANE L[1], GERBAUD L[1], BATEL V[1], BOUTELOUP C[1], BESSON G[2] AND COURATIER P[3], ON BEHALF OF THE GROUPE FRANÇAIS DES MALADIES DU MOTONEURONE

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Background: Tube-feeding's (TF's) effects on survival in ALS patients appear conflicting in the literature. No study has yet assessed TF's effects on quality of life (QoL); moreover, the best moment for initiating TF in ALS is still debated regarding both QoL and survival.

Objective: Assessing TF's effects on QoL and survival in amyotrophic lateral sclerosis (ALS) patients.

Methods: A prospective observational study was performed in 16 French teaching hospitals from January 2003. Patients were assessed every 3 months recording notably QoL (MOS-SF 36 and ALSAQ), anthropometry, ALS-FRS scales and respiratory function. Both QoL and survival were compared between patients accepting or refusing TF, and among those accepting we assessed the influence of weight loss on survival. QoL was studied as longitudinal data using a mixed model; survival time was analysed using Kaplan-Meier estimator, homogeneity tests and hazard ratios (HR) with 95% confidence limits were computed using the Cox model.

Results: We recruited 383 patients: 170 female/213 male; 61±12 (SD) years-old; 293 spinal/90 bulbar. TF was proposed in 115 patients notably for weight loss, prolonged meals, swallowing disturbances, motor incapacity. Sixty-three refused because of anxiety, a wish to delay or definitive refusal.
Regarding QoL dimensions, when accepting TF, patients experienced a favourable trend in ''energy'' (p=0.15) during the 6 months following TF. Considering time course evolution of ''social welfare'', a trend toward transitory improvement during the 6 months after TF was noticed in patients accepting TF (p=0.06).
There was no difference in survival time from TF indication regarding TF acceptance at first proposal (p=0.44).
Regarding weight loss from inclusion at first proposal, the mortality adjusted on sex and ALS initial clinical form was significantly lower in patients with a first TF indication before 10% weight loss. The hazard ratio of patients with late TF indication was 1.70 $((0.21; 2.01)_{95\%CL}$, p=0.03) as compared with patients having earlier TF indication.

Conclusions: These results suggest some transitory positive effects of TF on QoL and also show some survival benefit of early practiced TF, i.e. before 10% weight loss from weight at inclusion.

C93 NUTRITION MANAGEMENT BY DIETITIANS OF ALS/MND PATIENTS: A SURVEY OF CURRENT PRACTICE IN ENGLAND, WALES, NORTHERN IRELAND AND CANADA

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Background: Nutrition is an integral component of ALS/MND care. Evidence based nutrition guides exist but their use among registered dietitians is unknown. The aim of this study was to survey knowledge, practice and guideline use across England, Wales, Northern Ireland (EWNI) and Canada.

Method: Registered Dietitian (RD) contact details were obtained from the Motor Neurone Disease Association (MND Association) and ALS Society of Canada (ALSSC) websites. Telephone interviews were conducted with 23 dietitians using a standardized questionnaire.

Results: All RDs contacted (n=23) participated in the study (100%).
RD Service: Multidisciplinary team membership was high (78%). Case-loads varied widely (2-215). Most RDs (87%) worked part-time. Only 22% had more that 4 years

experience with ALS/MND care. Most (74%) saw patients in an ALS/MND outpatient clinic.
Knowledge & Sources of Information: Information on diet and nutrition information was obtained primarily from research papers. Just over half rated quality of information available as poor or adequate. Few RDs were familiar with existing guidelines and used them (23%).
Nutrition Assessment: Most or all RDs used body weight, % weight loss and BMI in assessing nutritional status. Few (9%) used mid-arm anthropometry and none used bioelectrical impedance or DEXA. Nutritional intake was assessed primarily using 24-hour diet history. Standard equations were used to estimate energy and protein requirements (Schofield and Elia respectively for EWNI; Harris-Benedict and DRIs respectively for Canada). Fluid requirements were estimated using a standard age-specific equation (Thomas).
Nutrition Treatment & Intervention: RDs were asked frequency of dietary advice in eight areas. Responses differed between the two groups but most frequent advice for both groups was high calorie, texture modification and nutrition products. Advice on weight reduction and creatine was given occasionally/never by both groups. Discussion of artificial nutrition and hydration (ANH) was rarely discussed at the first visit (26%). Triggers for discussion of ANH differed between the two groups. RDs in Canada used onset of dysphagia, weight loss of up to 10% and reduced FVC as indicators; RDs in EWNI used weight loss greater than 10%, onset of dysphagia and inadequate calorie intake as indicators. PEG tube care was offered by 78% of RDs. ANH by RIG and NGT was offered almost exclusively in EWNI.

Conclusions: The study showed a remarkable consistency in dietetic practice despite lack of awareness of published guidelines. Learning is experiential. RDs use knowledge from their dietetic training and expertise gained through working with this patient population. Discussion and collaboration between RDs and ALS/MND specialists at the international level is highly recommended. Adoption of current guidelines and development of standardized nutritional care is encouraged. The study also identified the need for further research on nutritional requirements e.g. energy, protein, vitamins/minerals, and publication of findings in dietetic journals.

C94 A RANDOMIZED, DOUBLE–BLIND, PLACEBO-CONTROLLED STUDY OF THE SAFETY AND EFFICACY OF BOTULINUM TOXIN TYPE B (BTXB) FOR SIALORRHEA IN ALS

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Background: Based upon data from the ALS Patient Care Database, 50% of ALS patients report sialorrhea and treatment with anticholinergic medication is ineffective or poorly tolerated in up to 30% of patients.

Objectives: The primary aim of this study is to determine patient perception of benefit of BTxb in ALS patients with medically refractory sialorrhea using a global impression of change scale. Secondary aims include establishing the safety of the injection paradigm, determining the effect of BTxb on salivary production and viscosity, and determining both patient and caregiver perception of benefit in terms of its impact on quality of life.

Methods: Twenty ALS patients with sialorrhea refractory to medical therapy were enrolled in this double-blind, randomized study to receive either 2,500 U of BTxb or a pH matched placebo into bilateral parotid and submandibular glands, using electromyographic guidance. Injections included 0.1cc (250U) per site at two sites in each parotid gland and 0.15cc (750U) per site at two sites in each submandibular gland. Blinded assessments were performed at baseline and at weeks 1, 2, 4, 8 and 12 and included: ALS Functional Rating Scale, visual analogue scale of benefit and salivary viscosity, SEQOL-DW, global expression of change, saliva production measurement, and number of suctionings per day. The caregiver also completed a visual analogue scale of benefit, global impression of change and the ALS CARE caregiver form. Safety was monitored with a dysphagia visual analog scale, FVC measurements, and monitoring of weight. Following the double-blind portion of the study, patients are offered participation in an open label extension trial for 12 weeks. During the open label extension, follow-up visits and study assessments were similar to those performed during the double-blind portion of the study. Dose adjustments were allowed during this open label period up to a total of 5,000U.

Results: Patients receiving BTxb reported a global impression of improvement of 82% at 2 weeks compared to 38% receiving placebo ($p < 0.05$). This significant effect was sustained at 4 weeks. At 12 weeks, 50% of patients receiving BTxb continued to report improvement compared to 14% receiving placebo. There were no significant adverse events, including dysphagia, in the BTxb group and no significant increase in the rate of decline of vital

capacity. Analysis of the secondary outcome assessments will also be presented.

Conclusions: In ALS patients who experience sialorrhea refractory to pharmacologic therapy or who are intolerant of the side effects of anticholinergic medications, botulinum toxin type B therapy is safe and effective.

C95 CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF AN ALS POPULATION NON-COMPLIAN TO NIV

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Background: ALS survival depends on Non-invasive ventilation (NIV) and on its compliance. Difficulties in adapting ALS patients to NIV have been mainly attributed to the bulbar onset specifically due to excessive sialorrhea. However no controlled trial has established this relationship.

Objective: To clarify this issue.

Methods: We followed-up prospectively 73 consecutive ALS patients adapted to NIV from 2000 to 2005, excluding dementia and other co-morbidities. We compared demographic and clinical characteristics at admission, survival with NIV and total survival from symptom onset in the compliant (group 2) and non compliant patients (group1). All underwent: respiratory function tests and blood gases; nocturnal pulse oxymetry (NPO), amplitude of the motor response by phrenic nerve stimulation; needle electromyography of the right diaphragm; ALSFRS. For comparison of the groups we used t-test, Chi^2-test, and univariate and multiple Cox regression analysis when appropriate.

Results: Group 1 had 15 F (8 Spinal and 7 Bulbar) and 9 M (8 Spinal and 1 Bulbar ALS), Ratio F/M=1.6 with mean age (mean$\pm$SD) 60$\pm$10 years with an overall compliance lower than 70%. Group 2, included 19 F (12 Bulbar and 8 Spinal) and 31 M (6 Bulbar and 25 spinal) Ratio F/M=0.6 with mean age$\pm$SD 60$\pm$12 and compliance over 70%. Significant differences at admission between groups were found:form of onset, FVC, PImax, PImax/P0.1 and amplitude (X^2=7.24; p=0.007; t-Test; p=0.024; 0.006; 0.000; 0.028) respectively. We found a significantly higher proportion of bulbar women in Group 1 with significantly lower values of PImax, P0.1, PImax/P0.1, and amplitude (p=0.001;0.000;0.008;0.000). Total survival and survival with NIV was significantly reduced in Group 1 when stratified for sex and form (Log-Rank Mantel-Cox; p=0.023: 0.05). In multiple Cox regression analysis, adjusted for sex, the significant predictors of survival were the variables related to inspiratory muscle strength and the time to NIV; in the same procedure adjusted to type of onset, no significant predictors were found.

Discussion: The poorer prognosis of bulbar ALS women non compliant to NIV is related to respiratory muscle strength, but not the type of onset. Since lower respiratory muscle strength at this age in women is expected, it implies the need of early adaptation to NIV in this population of ALS patients. On the other hand, since NIV substitutes weakened inspiratory muscles, further studies should focus on NIV parameters setting in bulbar women.

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SESSION 11 JOINT CLOSING SESSION

C96 A WINDOW TO THE BRAIN: SIMPLE NASAL OLFACTORY MUCOSA BIOPSY AS A MEANS TO BIOASSAY/BIOMARKER NEURONS AND GLIA IN ALS PATIENTS.

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Optimization of therapeutic development in ALS is critical in ultimately discovering effective interventions. Many realize ALS is a heterogenous disease of various pathophysiological pathways, which may differ among patient populations. Subdividing patients- based on pathophysiology- and/or drug responsivity has been a very successful approach to parceling new therapeutics in other medical fields such as cancer. Oncologists have the advantage of being able to biopsy tumors, and biologically and molecularly assess disease in patients. This approach has not been possible in ALS- except for the limited analysis of CSF. In order to better evaluate ALS patients, in terms of sampling neural tissue and evaluating the response of tissue to potential disease modifying drugs, we employed a simple outpatient nasal olfactory epithelium biopsy procedure. The advantages of nasal olfactory biopsy include: 1) well established, quick and reproducible outpatient ENT-based procedure, 2) tissue samples suitable for protein and RNA analysis, 3) tissue can be cultured, separating neuronal and glial subtypes, 4) neural stem cells can be propagated 5) repeatable procedure in the same patient is possible over time to assess response to drugs. In evaluating this tissue we discovered that astroglial-like cells, expressing various astroglial specific proteins, including GFAP and EAAT2/GLT1, were present in all olfactory biopsy specimens. The presence of astroglial-like cells (sustentacular cells) in this human tissue provided the first opportunity to study these living cells from ALS patients and their response to astroglial modifying drugs. We have now successfully carried out more than 20 biopsies in normal controls, and with collaborators, another 40 patients with other disorders. Our initial studies demonstrate highly stable and reliable expression patterns of the astroglial markers in both rodent and human olfactory tissues. Furthermore, in rodent studies, we can demonstrate that peripherally administered drugs can activate both brain/spinal cord astroglial genes/proteins as well as olfactory mucosa genes/proteins. Thus the nasal biopsy procedure may be a valuable approach to evaluate drugs targeted for astrocytes in ALS patient during the course of clinical interventions. A trial based on nasal biopsy sampling is planned for Europe in 2007. Baseline investigations of cultured astrocytes and neurons from olfactory epithelium biopsy samples will be discussed.

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C97 NEW APPROACHES TO THERAPY DEVELOPMENT WHICH CAN BE EXPLOITED IN ALS/MND

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Amyotrophic Lateral Sclerosis (ALS) is an incurable neurodegenerative disease marked by motor neuron cell death and muscle atrophy and remains a significant challenge for drug discovery and development. Recently a number of significant advances have been made which offer hope for the development of novel therapeutics in the Neurosciences and ALS in particular. The processes of neurodegeneration and neuroregeneration have been extensively dissected such that we now understand, in significantly more detail, the biochemical processes leading to neuronal loss and, importantly, some points at which this neurodegeneration can be halted and even reversed. In this overview I will focus on the science of drug discovery and focus on a number of approaches targeting muscle and nerve that appear most promising.

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THEME 1 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P1 USING A ''N OF 1 RANDOMIZED CONTROLLED TRIAL'' TO PROMOTE SWALLOWING FUNCTION IN A PATIENT WITH ALS AND COGNITIVE IMPAIRMENT

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Background: A 77 year old male with rapid onset bulbar ALS, cognitive dysfunction, and behavioural problems experienced frequent severe choking episodes and an aberrant pattern of inhaling after swallowing. He refused a PEG tube and was non-compliant with his recommended diet texture. He often exhibited angry outbursts while eating and his wife said he ''was in agony'' during most meals.

Objectives: The goal of this study was to promote expiration following swallowing to reduce the risk of aspiration and choking and to decrease anxiety during meals.

Methods: As described by Guyatt, Jaeschke and McGinn (1), a ''N of 1 RCT'' involves one patient who undergoes pairs of treatment periods such that one period of each pair is the experimental treatment and one period is an alternative or placebo treatment. The order of the active and placebo treatments is randomly assigned. In this study, the active treatment was explicit instruction in proper swallowing posture (i.e., open expanded chest) and the placebo treatment consisted of laryngeal adduction/voicing exercises. Each treatment period consisted of one session in which 10 trials of each treatment were performed. Two pairs of treatment periods were conducted (Placebo – Active – Active – Placebo). The primary outcome measure of interest was respiration during the swallowing act. Multiple assessment measures were administered prior to the initiation of the study and after each treatment period. While the patient swallowed liquids, the investigator assessed the respiratory cycle using a Vernier, 3-axis accelerometer and a respiration monitor belt. A secondary outcome measure was the patient's perception of problems during eating. The researcher used a subsection of the Swallowing Quality of Life Scale (2) in which the patient rated the most troubling symptoms he experienced during mealtimes (e.g., frustration, annoyance, impatience, and discouragement) on a five-point Likert scale (1=frequent or severe problems; 5=no problems).

Results: Prior to the study, the patient demonstrated an aberrant pattern of swallowing with inspiration. The typical pattern of swallowing during expiration was observed in only 13.8 % of trials on average. During the study, the placebo treatment resulted in the typical and desired pattern of expiration after swallowing in 7.7% of trials on average whereas the active treatment resulted in this pattern in 76.4 % of trials on average. With regard to the patient's perceptions of problems during eating, the patient's average baseline rating of distress during meals was 1.5/5. After the placebo treatments his average ratings were 1 and 1.6. After the active treatment, his ratings were 2.6 and 2.8.

Discussion and conclusions: This patient learned a compensatory swallowing technique and exhibited a more typical swallowing-respiration pattern as a result of treatment. The patient's swallowing-respiration pattern did not change significantly as a result of the placebo treatment. The patient rated his psychological distress as less frequent/severe following the active treatment as compared to placebo. The interventions were designed to capitalize on intact oral pharyngeal sensation and capacity to learn, despite cognitive deficits.

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P2 THE NUTRITIONAL STATUS OF PATIENTS WITH ALS – A PROSPECTIVE STUDY USING BIOELECTRICAL IMPEDANCE ANALYSIS

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Background: Amyotrophic lateral sclerosis (ALS) is an incurable degenerative progressive disease. Pharmacological treatment of patients suffering from ALS is limited to riluzole, which has shown to be the only drug to prolong life expectancy. The primary task of the treatment during the course of disease is to develop symptomatic strategies. The nutritional status is of essential interest since there is evidence that patients with ALS have underlying altered energy metabolisms.

Objectives: The bioelectrical impedance analysis (BIA) is an established method to obtain information on the composition of body compartments. The aim of this study was to analyze several BIA parameters in a longitudinal follow-up design, which reflect information on malnutrition in ALS.

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Methods: Fifty-four consecutive patients with possible or definite ALS according to revised El Escorial criteria were recruited from the outpatient clinic for MND of the University of Ulm and underwent BIA analysis at study inclusion, and after 3, 6 and 9 months. At each point of time all patients received additional clinical investigations, measurements of body weight and performance of the revised ALS-FRS.

Results: There was a decrease in body weight in male patients and body mass index (BMI) in all patients. The values of the ALS-FRS were in a continuous decline from study inclusion to month 9. BIA revealed a decrease in the basic metabolic rate (GU), the body cell mass (BCM), the phase angle (PA) and the fat free mass (FFM). There were significant correlations both of PA and ALS-FRS and GU and ALS-FRS.

Conclusion: BIA is a stable and reliable method for the objective analysis of the nutritional status of ALS patients and might be a useful tool for a well-timed initiation of supportive feeding protocols in order to maintain functional capabilities.

P3 NUTRITIONAL FOLLOW-UP OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: A significant problem for patients with amyotrophic lateral sclerosis (ALS) is maintaining adequate nutrition. The symptoms and progression of the disease can affect the patients' nutritional status leading to weight loss and malnutrition. Nutrition is an independent prognostic factor for survival but it is not known how much nutritional intervention is needed.

Objectives: To evaluate the patients' nutritional status and nutritional problems during the follow-up and the effects of individualized nutritional therapy.

Methods: A nutritional evaluation was performed in all ALS patients consecutively referred to our tertiary level ALS centre. The assessment included: ability to eat, chew and swallow; weight loss; food intake using a 24-h alimentary record; anthropometry: height, weight and body mass index (BMI=weight [kg]/height [m]^2), triceps skinfold thickness, mid-upper arm circumference and mid-upper arm muscle area (MAMA); evaluation by a speech therapist in the presence of swallowing disorders. Nutrition counselling and/or individualized nutritional therapy was provided to all patients and carers. Patients were followed-up at 3-4 monthly intervals: nutritional assessment was performed at every visit.

Results: 63 patients (34 males, 29 females) were regularly followed-up for an 18-month period. Mean baseline values (T0) were: age 52.6 years (range 20-79); disease duration 14 months (range 1-60); bulbar involvement in 31.7 %; weight loss compared to usual body weight in 26.9 %; difficulties in autonomously eating, chewing and swallowing respectively in 42.9 %, 23.8 % and 50.8 %; BMI 24.9 ($<$18.5 in 11.5%, 18.5–24.9 in 47.6%, $>$25 in 40.9%); MAMA below 5° percentile in 52.1%; calorie intake 1945$\pm$485 Kcal/die; nutritional therapy: diets modified in consistency (17.4%), diets modified in calorie and protein content (11.1%), thickening powder (4.7%). During the 18-months follow-up we observed: difficulties in autonomously eating, chewing and swallowing respectively in 69.8 %, 41.3 % and 60.4 % of cases; BMI at 6 months 23.9 ($<$18.5 in 13.0%, $>$25 in 30.4%), at 12 months 23.8 ($<$18.5 in 11.7%, $>$25 in 33.3%), at 18 months 22.9 ($P<0.05$ vs T0) ($<$18.5 in 17.6%, $>$25 in 35.3%); MAMA below 5° percentile in 84.6%; caloric intake 1838$\pm$335 Kcal/die; nutritional therapy: nutritional high calorie supplements (14.3 %), diets modified in consistency (38.1%), diet modified in calorie and protein content (23.8%), thickening powder (19.0%), enteral nutrition after percutaneous endoscopic gastrostomy (PEG) placement (15.8 %).

Conclusions: The progression of ALS affects the patient's nutritional status, limiting feeding ability (autonomous eating, chewing and swallowing difficulties), leading to progressive weight loss. Regularly performed nutritional assessment and early individualized nutritional interventions can limit the nutritional deterioration and aid in the treatment of the disease. During the 18-months follow-up period 60.3% of patients received a nutritional therapy and, despite the progression of the disease, 47.1% of patients maintained a normal BMI value.

P4 ENTERAL AND PARENTERAL NUTRITION IN THE LATER STAGES OF ALS

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Background: Nutritional management is a great concern in patients with ALS. Current practice guidelines state that enteral feeding should be offered and placed when there is a significant dysphagia or weight loss. There is evidence that enteral feeding improves nutritional status in patients with ALS. Evidence for other benefits, including effects on survival and on quality of life has not been proven. Percutaneous endoscopic gastrostomy (PEG) is usually offered in ALS centres but is not indicated in patients with severe respiratory impairment. Moreover, tube feeding is sometimes refused by the patient or impossible.

Objective: To evaluate the feasibility of home parenteral nutrition (HPN) in ALS patients with respiratory insufficiency or refusing feeding tube, to assess the complications and survival of ALS patients after the procedure and to compare the results with those of patients with PEG.

Method: HPN, performed through an implanted port, was proposed to ALS patients requiring nutritional support with respiratory insufficiency (patient with NIV or vital capacity $<50\%$). HPN was proposed too if tube feeding was refused or impossible. Thirteen patients (8 female, 5 male, age 48–79 yrs) agreed to HPN and were included over a 12 month period.
Nutritional and neurological follow-up was performed. Complications and survival were prospectively studied. We compared these results to those of a group of 23 patients with PEG (age 37–82 yrs) with subgroups including patients with (n=7) vs without respiratory insufficiency (n=16) at the date of the PEG. Spearman test was used for statistical analysis.

Results: Weight was stabilized in 11 patients with HPN and weight gain occurred in 2 others. Complications occurred in 2 patients with PN : early bacterial infection and hematoma complicated the implantation of the port in one case; delayed bacterial infection occurred in the other case. Survival after procedure was from 1 to 10 months, 11 patients died from respiratory failure, 1 patient died from acute sub-dural hematoma. For one patient death was directly related to the early complication due to the port. Post-procedure survival was comparable between patients with PEG and respiratory insufficiency and patients with HPN ($4\pm3{,}3$ months vs $3{,}4\pm2{,}9$, NS). In the group of patients with PEG performed without respiratory insufficiency, survival was better ($14\pm9{,}2$ months).

In the group with PEG, minor early complications occurred in 2 patients (infection, haemorrhage); major complications occurred in two other patients leading to death (haemorrhage and peritonitis).

Conclusion: The results of this study suggest that HPN can be proposed in patients with advanced ALS, and could be an alternative to enteral feeding.
HPN is safe and effective and improves nutritional status with stabilization of weight. Survival rate in months was similar to patients with enteral feeding at the same stage of disease.
Further resarch is needed to assess the benefit of HPN in the end of life care of ALS patients and to determine the impact on patients' and caregivers' quality of life.

P5 PATIENTS SUFFERING FROM AMYOTROPHIC LATERAL SCLEROSIS WITH PEG: NUTRITIONAL ISSUES

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Background: Patients with Amyotrophic Lateral Sclerosis (PALS) are often faced with progressive nutritional problems leading eventually to percutaneous endoscopic gastrostomy (PEG) placement. Due to the disease itself, and in particular the novel clinical condition with a feeding device, PALS experience psychological distress which impairs their quality of life.

Objectives: To evaluate nutritional state in PALS with PEG.

Methods: We evaluated 11 spinal-onset (s-o) or bulbar-onset (b-o) PALS (6 men and 5 women; mean age $\pm$ SD: 63 ± 11 yrs) with PEG. Various nutritional data (weight, body mass index, blood prealbumin, albumin, transferrin and lymphocyte count, body cell mass index (BCMI) and other soft tissue analysis parameters) were collected just before and 2 months (t_2) after PEG insertion, and correlated with disease severity (according to the ALS Functional Rating Scale, ALSFRS). In a subgroup of 7 patients (4 men and 3 women; mean age $\pm$ SD: 63 ± 8 yrs), we studied nutritional data also after 8 months (t_8) from PEG insertion.

Results: By comparing the 11 PALS before PEG insertion (mean ALSFRS score $\pm$ SD: 16 ± 9) and at t_2 (mean ALSFRS score $\pm$ SD: 11 ± 7), we found BCMI mean values significantly ($p<0.05$) reduced after PEG placement (5.6 ± 2.6 vs 4.5 ± 1.4), while plasmatic prealbumin (25.5 ± 7.7 vs 27.1 ± 4.9 mg/dl) and serum albumin (4.6 ± 0.5 vs 4.7 ± 0.3 g/dl) increased, and blood transferrin and lymphocyte counts remained unchanged. In particular, significantly ($p<0.01$) enhanced mean values of serum albumin were observed in s-o patients (4.1 ± 0.5 vs 4.7 ± 0.4 g/dl).
Moreover, by considering a subgroup of patients evaluated at t_2 and t_8 (mean ALSFRS scores $\pm$ SD: 15 ± 9 vs 10 ± 6 vs 9 ± 6), we found reduced BCMI mean values overtime (5.7 ± 2.6, 4.5 ± 1.4, and 4.1 ± 1.7, before PEG insertion and at t_2 and t_8, respectively) together with increased plasmatic prealbumin (26.6 ± 8.7 vs 27.2 ± 6.3 vs 29.1 ± 5.1 mg/dl), serum albumin (4.2 ± 0.4 vs 4.5 ± 0.1 vs 4.6 ± 0.5 g/dl) and transferrin ($2{,}1\pm0.4$ vs 2.6 ± 0.3 vs 2.7 ± 0.2 g/l) mean values, whereas lymphocyte counts remained unchanged.

Discussion: Although the disease still keeps progressing (as shown by relentless BCMI reduction), our results support the concept that PEG placement can improve nutritional status of PALS patients (as shown by enhanced values of plasmatic prealbumin and serum albumin and transferrin).
The relevance of an interdisciplinary follow-up for ALS patients with severe nutritional problems will be thoroughly discussed.

P6 AN AUDIT OF THE USE OF GASTROSTOMY TUBES IN PATIENTS WITH MOTOR NEURONE DISEASE

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Background: The use of gastrostomy tubes in patients with motor neurone disease is recommended in patients with difficulty maintaining good nutrition due to pronounced dysphagia. However the timing and type of gastrostomy tube is not established. The MND Association (1) and Chio *et al* (2) have suggested a range of criteria that may be used to determine the timing of

gastrostomy tube insertion. Percutaneous radiologic gastrostomy (PRG) tubes have been proposed as a safe and effective method of nutritional tube placement in MND (3) and as an alternative to percutaneous endoscopic gastrostomy (PEG) tubes.

Objectives: The primary aim of this study was to review all patients seen in a regional MND clinic with a view to assessing whether patients who fulfilled the criteria for a PEG/PRG tube had this discussed. Secondly, to assess whether the morbidity and mortality of patients who had either a PEG or PRG tube inserted differed.

Methods: The data from all patients seen in a regional MND clinic over a period of one year from Jan 2006 – 2007 were retrospectively obtained. Data on the following criteria were extracted – weight loss of over 10%, severe dysphagia, inadequate energy intake, functional vital capacity of less than 50% of predicted, history of aspiration and a body mass index of less than 20.

Results: Data from 49 patients were analysed. The mean (standard deviation, SD) age at diagnosis was 62.7 (12.4) years. 67% of the patients were male. Majority of the patients had limb presentation (57% compared to 30% bulbar and 13% both). Gastrostomy tubes were discussed in 50% of the patients and in over 60% of the patients with any of the criteria above. Almost half of the patients (46%) who had a gastrostomy tube advised were not keen at initial discussion. In 29% of these patients, it was not documented that a gastrostomy tube may not be applicable as the disease progressed. Of the total 49 patients, 20 (42%) had a gastrostomy tube placed – 13 had a PEG and 7 had a PRG. There was no significant difference in the complication rate but patients with PRG had a longer hospital stay (PEG v PRG - 3.9 days v 9.2 days, $p=0.086$). 5 patients with a PEG and 2 with a PRG tube have died to date. There was no significant difference in median survival between the two groups – both being 7 months, $p=1$.

Conclusions: Comparing our results to the guidelines suggested by the MND Association and Chio et al, the majority of patients who had at least one of the criteria for a PEG/PRG did have this discussed. However the results of our practice did not find that PRG tubes conferred a significant advantage over PEG tubes.

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P7 PERCUTANEOUS ENDSCOPIC GASTROSTOMY UTILIZING 3D-CT IMAGES: FOCUS ON AMYOTROPHIC LATERAL SCLEROSIS WITH RESPIRATORY MUSCLE WEAKNESS

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Background: In amyotrophic lateral sclerosis (ALS) with dysphagia, percutaneous endoscopic gastrostomy (PEG) may provide a survival benefit. Quality of life also may be improved. However, due to the potential hazard of PEG for PALS with respiratory muscle weakness, PEG is only recommended when the percent predicted forced vital capacity (%FVC) exceeds 50%. On the other hand, some patients do not consider having a PEG tube placed before deterioration of their respiratory status to a critical level, and by the time the decision is made, respiratory muscle strength reduction has already progressed with %FVC lower than 50%.

Objective: In ALS with respiratory muscle weakness, while it is desirable to execute PEG tube placement in the shortest time possible, surgical complications including mispuncture of the colon or abdominal aorta should not occur. Until now, safety precautions including abdominal plain radiograph with infusion of air into the stomach, abdominal CT, fluoroscopic monitoring, confirmation of finger sign (impression of the finger pressing over the abdominal wall is clearly depicted as submucosal tumor-like object under endoscopic observation) have been recommended, but are not adequate. We examined whether safety of the PEG is improved by using 3D-CT imaging.

Methods: Nine patients with ALS or other neurological disease who had dysphagia necessitating gastrostomy were studied. Excluding 3 patients who had tracheostomy, the FVC was 18.8-102.1% in the remaining 6 patients. Before and after PEG, abdominal multi-row (8 rows) detector computed tomography (MDCT) was conducted using Light Speed Ultra (GE Yokogawa Medical Systems) after injecting 500 mL of air into the stomach. Using volume rendering, continuous 3D images of slices with even thickness were constructed, and reconstructed images that depict the profile of the internal gastric wall surface were fused. A 3D processing workstation equipped with ZIO-M900 Quadra (AMIN) was used.

Results: Using abdominal 3D-CT image as reference, the appropriate site of entry and the positional relation between the colon or abdominal aorta and the stomach are known before surgery, and PEG was conducted rapidly and safely. In all cases, the PEG procedures from endoscopy insertion to completion took 5-10 min. Even cases with FVC $\leqslant$30% had uneventful courses with no postoperative complications including pneumonia.

3D-CT after PEG permitted visualization of the state of the gastrostomy and detection of complications such as buried bumper syndrome. During changing of the PEG tube, detection of problems such as long fistula and oblique insertion was useful to decide intervention policies such as changing under endoscopic monitoring.

Conclusion: In ALS patients with respiratory muscle weakness, evaluation of PEG insertion site using 3D-CT imaging contributes to safe PEG procedures.

P8 CO_2 CHANGES INDUCED BY ENTERAL NUTRITION IN MECHANICALLY VENTILATED PATIENTS

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Backgrounds: End-tidal partial pressure measurements of carbon dioxide ($P_{ET}CO_2$) relate to partial arterial pressure measurements of CO_2 ($PaCO_2$) and have been widely applied in intensive care treatment settings and during surgical operations. This methodology is useful in the respiratory care of amyotrophic lateral sclerosis (ALS) patients.

Objectives: To elucidate a respiratory management problem by blood gas examinations and to evaluate the usefulness of monitoring of $P_{ET}CO_2$ in mechanically ventilated patients.

Methods: Three patients with mechanical ventilation (ALS and Sjogren syndrome) were investigated. Enteral nutrition was administrated three times a day in each subject. A $P_{ET}CO_2$ sensor kit (Nihon-Koden, TG900P) was attached to a respirator circuit and changes in $P_{ET}CO_2$ per hour were measured consecutively over several days. In one case, changes in $P_{ET}CO_2$ were evaluated during intravenous hyperalimentation (IVH).

Results: $P_{ET}CO_2$ increased soon after the onset of enteral nutrition and recovered four hours later. $P_{ET}CO_2$ measurements were at a minimum early in the morning in all three cases. The ALS case had diabetes and a postgastrectomy as a part of the medical history, and $P_{ET}CO_2$ showed significant changes (31.0 to 47.4 mmHg) within the day. About a 10 mmHg change within the day was seen in the two other cases. $P_{ET}CO_2$ values from midnight to early morning were lower during the enteral nutrition than those during the IVH.

Discussion and conclusion: We supposed that $PaCO_2$ would remain constant while ventilation conditions remained unchanged. However, changes in $P_{ET}CO_2$ observed in this study were surprising. Rises in $PaCO_2$ by internal CO_2 production following enteral nutrition in situations where constant ventilation was provided have been reported (1,2). The importance of the respiratory exchange ratio (3), and an increased $PaCO_2$ (26.3 to 34.4 mmHg) on the day following the onset of enteral nutrition has also been reported (4). However a few blood gas tests do not seem to detect these significant changes in $P_{ET}CO_2$ throughout the day. Setting a respirator only by the blood gas test may cause hypoventilation or hyperventilation. Thus, since this methodology is simple, noninvasive and appropriate for long term monitoring, $P_{ET}CO_2$ measurements would be useful in the optimal respiratory care of ALS patients.

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P9 PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: ROLE OF BIPAP-VENTILATION.

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Background: Malnutrition is an independent prognostic factor relating to the survival of patients with amyotrophic lateral sclerosis (ALS). Percutaneous endoscopic gastrostomy (PEG) can help to solve this problem, even though patients with diminished lung function have an increased mortality after PEG placement. Procedure-related mortality can reach 1.8%, 24-hour mortality 3.6% and 30-day mortality 11.5%. The mean survival rate after intervention is approximately 200 days (185-211 days).

Objectives: We aimed to investigate if it would be possible to improve the outcome of PEG insertion in ALS patients, by noninvasive BiPAP-ventilation.

Methods: Retrospectively, we examined data of 29 ALS-patients in whom PEG was inserted with a pull-through method (Freka® PEG Set Gastric Fr 15) between March 2002 and August 2006. Median age of the patients was 63 years (range 32-83). Median time between diagnosis and PEG-insertion was 25 months (3-108), median BMI 22.2 kg/m2 (14-26). Median forced vital capacity (FVC) was 32.5% of normal. In 12 patients FVC was less than 50%. Patients with diminished pneumological parameters (low FVC, reduced ability to cough, low SaO2 , low sniff nasal

inspiratory pressure) were classified in three different risk groups and instructed to BiPAP-ventilation during a period of one week. BiPAP-ventilation was performed by means of a special mask. The endoscope was inserted through a particular hole in the mask, so that the oxygenation and ventilation of the patient was not interrupted during the examination.

Results: We did not observe any death cases during endoscopy and PEG insertion with BiPAP-ventilation (24-hour mortality 0%). We didn't have any severe complications, although one patient had to be intubated 3 hours after the intervention. She survived 8 months thereafter. The overall 30-day mortality was 3.4% (n=1) and the median survival time was 240 days (18-960). Up to now, 9 patients are still alive.

Conclusions: BiPAP-ventilation impacts positively on post-interventional mortality during PEG placement in patients with ALS and impaired respiratory function. A thorough instruction to BiPAP-ventilation is therefore highly recommended.

P10 TWO NIGHT POLYSOMNOGRAPHIC STUDY IN MND / POST-POLIO SYNDROME PATIENTS

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Background: The Post-Polio Syndrome (PPS) is caused by muscle overuse during decades. The main MND/Post-Polio Syndome symptoms are new weakness, new atrophy, cold intolerance, fatigue, pain and sleep disturbances. The polysomnographic study is the gold standard to analyze sleep disturbances. The main sleep disturbances are obstructive sleep apnea (OSA), characterized by upper airway obstruction due to pharyngeal muscles weakness, and periodic limb movements (PLM), characterized by stereotyped leg movements during sleep, which causes sleep fragmentation due to micro arousals, decreases in sleep efficiency, and sleepiness.

Objectives: We aimed to assess whether a two-night sleep study is adequate in the assessment of sleep disturbances in PPS patients.

Participants and settings: Thirty patients with PPS (21 women and 9 men), mean age 46,1 ± 9,2 years old, from the Federal University of São Paulo – Neuromuscular diseases sector. We did a descriptive study including: medical and physical evaluation and consecutive two-night polysomnographic study.

Results: In both nights, the sleep efficiency decreased. There was a significant decrease in REM sleep latency (p=0,009) from the first to second night. The breathing events (apneas and hypopneas) were mainly obstructive in both nights, but there was no statistical significance. The PLM index was moderate in both nights, also without statistical significance.

Conclusion: As we had shown and discussed previously, there's no need to make a second-night polysomnography exam in PPS patients.

P11 NOCTURNAL HYPOXIA IN EARLY MOTOR NEURON DISEASE IS NOT PREDICTED BY STANDARD RESPIRATORY FUNCTION TESTS

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Background: With increasing awareness of motor neuron disease (MND), the approach to respiratory management of patients with this disease will more commonly face the physicians who manage MND.
While respiratory failure in MND patients is generally a direct consequence of muscle weakness during disease end-stage (1), a proportion of patients develop early and severe respiratory difficulties during sleep, termed nocturnal hypoventilation. Hypoventilation produces nocturnal hypoxia (NH), which is becoming increasingly recognized as a predictor of survival, independent of respiratory muscle weakness (2,3). It has recently been demonstrated that intervention with non invasive ventilation in MND patients with symptomatic respiratory failure improves survival (4).

Objective: The aim of this study was to determine if standard respiratory function tests could determine the presence of NH in MND patients without respiratory symptoms.

Methods: Respiratory function tests were used to examine daytime respiratory function and sleep studies were used to detect NH in 16 consecutive patients with MND, as well as nine healthy control subjects. Demographic data, clinical parameters, respiratory function tests and oximetry studies were obtained. Statistical analyses were performed using t-tests and analysis of variance where appropriate.

Results: NH was detected in 50% of patients with MND with no hypoxic events detected in the control group. Standard respiratory function tests were not able to predict the presence of NH.

Conclusion: There was no correlation between respiratory function test parameters and the presence of NH. This study highlights the inability of standard respiratory function tests to predict nocturnal hypoxia that may arise early in the course of motor neuron disease.

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P12 ASSESSMENT OF SNIFF NASAL INSPIRATORY PRESSURE (SNIP) IS USEFUL IN PREDICTION OF PROGNOSIS IN JAPAN

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Background: We have investigated the usefulness of SNIP in Japan by cross-sectional study. The previous study confirmed reliability and validity, and showed that SNIP values in Japanese people were lower than in Caucasian people (1). However, it was unclear about both method of SNIP measurement in ALS patients with bulbar symptoms and the relationship between SNIP and prognosis in Japan.

Objects: The purposes of this study were 1) to examine methods of SNIP measurement in ALS patients with bulbar symptoms, 2) to investigate usefulness in prediction of prognosis longitudinally in Japan.

Methods: Fifty-seven ALS patients (average age: 64.1 ± 10.0 years, average disease duration: 2.3 ± 2.3 years) were enrolled in this study, and SNIP measurement was executed continuously. For SNIP measurement, the plug was inserted into the nostril and contralateral nose was not occluded (opening nose method). Patients with bulbar symptoms with a SNIP value of less than 30 cmH_2O were measured by another method too, in which contralateral nose was occluded (occluding nose method). Based on SNIP data that were collected continuously, the average of change quantity per one month was calculated. To examine the relationship between SNIP and prognosis, a logistic regression model was used.

Result: In the patients with bulbar symptoms with decreasing SNIP, the SNIP value measured by occluding nose method was significantly higher than that of opening nose method($P < 0.01$). There were patients for whom SNIP could not be measured continuously by the opening nose method, but all the patients could be measured by the occluding nose method.

During the investigation period, sixteen patients were managed by tracheotomy positive pressure ventilation (TPPV) or died. Logistic regression model showed that only average of change quantity was related to prognosis (death or TPPV management) significantly, that was independent from age, disease duration, follow up phase, bulbar involvement and baseline SNIP value. When SNIP was decreasing more than 2 cmH2O/month, hazard of death or TPPV management rose significantly (Kaplan-Meier survival curve and log rank test).

Discussion and conclusion: Our findings showed that, by the use of occluding nose method in ALS patients with bulbar symptoms, sensitivity of SNIP measurement could be improved, and change of respiratory function could be observed for the long term.
Morgan et al reported that SNIP value at baseline was related to prognosis, but our study showed that continuous change quantity of SNIP was significantly related to prognosis in Japan. Therefore, it was thought that continuous decrease of SNIP had a higher death or TPPV management risk than decreasing SNIP at baseline in Japan.

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P13 MEASURING RATE OF DECLINE IN PULMONARY FUNCTION IN ALS: PRELIMINARY RESULTS FROM THE ALS NUTRITION/NIPPV STUDY GROUP

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Background: Objective measurements of respiratory muscle function are used to determine the timing of noninvasive ventilation (NIV) and prognosis. While forced vital capacity (FVC) is currently regarded as the standard indicator of disease progression, other measures may provide a more sensitive indicator of respiratory muscle dysfunction than FVC to direct institution of NIPPV.

Objective: To determine which pulmonary measure is the most sensitive measure for determining a decline in respiratory function and to determine whether criteria for initiation of NIV were met first by FVC or by inspiratory pressures (IP).

Methods: As part of a large multi-center study of NIV and nutrition collection of serial pulmonary function measures are being performed over a 12 month period on subjects with definite or probable ALS. We compared serial pulmonary function measurements of maximum voluntary ventilation (MVV), maximum inspiratory pressure (IP) measured by mouth (MIP) or nasal sniff pressure (SNP), maximum expiratory pressure (MEP), and FVC to determine which measure showed the fastest decline in pulmonary function Because of known effects of position on diaphragm function, SNP and FVC were also measured in the supine position. FVC and MVV were measured by spirometry (Respironics Renaissance). Pressure measurements were measured using a portable electronic pressure meter (MicroMedical). The best value of 3 efforts was used for analysis. Rate of decline of pulmonary function for each measure was determined as the mean slope of the percentage drop from the initial baseline value over time. NIV initiation criteria were considered met if FVC was $\leqslant 50\%$ of predicted or IP was $\leqslant 60$ mmHg.

Results: To date there have been 54 patients enrolled in the study with a median age of 60 years. Sixty-five percent of the participants are male and 35% are female. Forty-five subjects who had at least 2 visits were analyzed for decline in respiratory function (median follow-up 167 days, range 49-313). Over the interval measured, only three of the mean slopes were significantly different from zero: MEP (-0.190 %/d), sitting FVC (-0.120%/d), and supine SNP (-0.113 %/d). A total of 54 patients with at least one visit were analyzed for NIV initiation criteria. 47 patients attained the IP criteria first; 37 of these met criteria at their baseline visit. 2 patients attained the FVC and IP criteria simultaneously on the same visit. Only 1 patient met FVC criteria first.

Conclusions: a) Preliminary data suggest a more rapid decline in MEP than other measures, suggesting that there is early and more rapid deterioration of expiratory muscle strength. b) Criteria for initiation of NIV were more easily met using inspiratory force criteria, suggesting that IP is either a less stringent criteria or that it is a more sensitive measure of respiratory impairment.

P14 PULMONARY FUNCTION TESTS AND PHRENIC NERVE CONDUCTION STUDIES IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background : Respiratory distress may be life-threatening while the neurological disorders are still limited. Neurophysiological assessment of breathing is mandatory for the diagnosis and management of respiratory problems occurring in Amyotrophic lateral sclerosis (ALS).

Objective: To evaluate the Pulmonary functions (PFT) in patients with ALS and to correlate with Phrenic nerve conductions (PNC).

Methods: Prospective study: Twenty nine patients (M-20, F-9) studied were diagnosed with ALS by El Escorial criteria. Thirty age and gender matched controls were selected from patients' relatives. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow rate (PEFR) and maximum voluntary ventilation (MVV) were measured by spirometry. Maximum expiratory pressure (MEP) was measured by digital peak pressure monitor. None had symptoms of pulmonary dysfunction, and were able to perform the PFT satisfactorily. The latencies and amplitude of diaphragmatic compound action potential (DCMAP) was recorded in ALS patients.

Results: The mean age of patients was 51.41yrs and control was 53.57yrs. No patient had clinical evidence of respiratory dysfunction. The FVC, FEV1, PEFR, MVV, MIP and MEP were significantly ($p < 0.001$)reduced in ALS. The mean amplitude was $610 \pm 506.231 \mu v$ and mean latency was 9.73 ± 2.57 ms. There was negative correlation between PFTs and latencies of PNC($p < 0.05$) and no correlation between amplitude, latency of PNC with age, height and weight.

Conclusion: This study suggests that patients with ALS have subclinical respiratory dysfunction due to weak respiratory musculature. There is significant negative correlation between PNC and PFTs suggesting early loss of myelinated fibres and diaphragmatic dysfunction.

P15 MOTOR POINT DIAPHRAGM PACING IN PATIENTS WITH MND/ALS: LONG TERM FOLLOW-UP OF COMPLETED SAFETY AND FEASIBILITY STUDY

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Background: Respiratory insufficiency is the major cause of mortality in patients with ALS. Ventilators, although life-saving, are inconvenient and associated with significant risks and alternate therapies to prevent or manage respiratory muscle decline in ALS are needed. The motor point diaphragm pacing stimulation (DPS) system has become a standardized minimally invasive laparoscopic technique providing ventilation in spinal cord injured patients. This report outlines the results of the application of DPS in ALS since the first implantation in 2005.

Objective: Analyze safety, utility and long term use of the DPS system from the initial FDA single site study.

Methods: Patients underwent outpatient laparoscopic diaphragm motor point mapping with electrode implantations. Stimulus/output characteristics of each electrode were determined and diaphragm conditioning initiated. Patients conditioned their diaphragms with 5 daily stimulation sessions of 30 minutes each but were allowed to increase usage. Each patient had three extensive lead-in assessments that were continued post implantation of the DPS system and included pulmonary function tests, fluoroscopic evaluation of diaphragm movement, speech phonation times, ultrasound analysis of diaphragm thickness, and quality of life tests.

Results: Sixteen patients were implanted with no adverse events with 7 undergoing simultaneous feeding tube placement. Average age was 50 (range 32-70) with 13 males. Average of 35 months of symptoms until enrolment with an ALSFRr of 26 at surgery. The average predicated forced vital capacity (FVC) at surgery was 52% with 12 patients eventually having bulbar symptoms. In all patients, more fluoroscopically observed diaphragm excursion occurred with diaphragm stimulation than under maximal voluntary effort. DPS significantly increases muscle thickness when assessed with ultrasound (p-value 0.02). In a subgroup analysis, those patients with declining FVC and bulbar symptoms pre-operatively reached statistical significance going from pre-implant slope of -2.9 to -1.4(%FVC /month with $p < 0.05$) The first four implanted patients expired 16.5 months post implantation. Given that their average FVC was only 49% predicted at surgery this is an improved survival compared to historical data. The cause of death was secondary to a fall, aspiration, peri-operatively from a cervical spinal fixation with only one being respiratory after a brief trial of mechanical ventilation. Additional study findings include: DPS can convert fast twitch glycolytic (IIb) to functional slow twitch oxidative muscle (I) fibers; DPS improves posterior lobe lung ventilation; DPS increases lung compliance leading to decreased work of breathing; and 7 patients utilize DPS to improve night-time ventilation.

Conclusion: The diaphragm pacing system can be safely implanted and utilized in patients with ALS with over 15 years of cumulative use. There has been a documented decrease in the decline of respiratory failure which leads to an increased survival. The ability to specifically target and improve diaphragm function with the DPS system will increase therapeutic options in these patients with specific focus on night-time sleep dysfunction. A multi-center pivotal trial is now enrolling patients and collecting data.

P16 AVERAGE VOLUME ASSURED PRESSURE SUPPORT (AVAPS) AS AN IDEAL INDICATOR OF INSPIRATORY POSITIVE AIRWAY PRESSURE (IPAP) IN ALS PATIENTS

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Background: Noninvasive intermittent positive-pressure ventilation (NPPV) may benefit ALS patients with respiratory insufficiency and AVAPS has been considered a strategy to permit a possible controlled volume in NPPV ventilators.

Objectives: To evaluate the AVAPS function as an indicator to determinate the ideal IPAP in ALS patients.

Methods: Ten patients with ALS, according to El Escorial criteria, using NPPV were followed, during six months. We measured: maximal inspiratory pressure (MIP), forced vital capacity (FVC), arterial oxygen saturation (SaO_2); exhaled carbon dioxide ($EtCO_2$), Epworth sleepiness scale and hypoventilation. We did BiPAP® download to evaluate the NPPV adesion, and the parameters (IPAP), Expiratory Positive Airway Pressure (EPAP), and tidal volume (TV) over the last three months. We changed the ventilation system (BIPAP to BiPAP with AVAPS) after one week, reajusting the TV between 8-10 ml/kg. All patiens were ventilated as spontaneous / timed (S/T) mode. After one week, BiPAP® with AVAPS download was done to find the ideal IPAP for each patient. The ideal IPAP was adjusted to the BiPAP® without AVAPS. After one week another BiPAP® download was made to evaluate if the TV was maintained. The same measures were done after 3 and 6 months. The level of significance was set at $p < 0.05$.

Results: The median age was 57.0 (66.0–45.5) years old, illness time 40.0 (51.5–34.5) months, time of evolution 30 (35.5–17) months and NPPV use 16 (19–8.5) months. The MIP was 18.0 (27.0–12.0)cmH_2O, started CVF was 40 (49–28.5)% , SaO_2 was 95 (96.0–94.0)%, $EtCO_2$ was 39 (40.5–38) mmHg, Epworth sleepiness scale was 3.0 (4.5–1.5), hypoventilation signs and NPPV hours 508.5 (728–320.5) minutes. The IPAP without AVAPS was 15.0 (21.0–13.5) cmH_2O and TV was 539.0 (590.0–452.5) ml, ($p < 0.0001$). Compared to IPAP with AVAPS of 20.5 (22–18) cmH_2O ($p < 0.0002$) and TV of 547.5 (668–482)ml ($p < 0.0001$) at start time (ST), after three months (T3) IPAP was 21(22-18)cmH_2O ($p < 0.0002$) and TV 564 (712.5–459)ml ($p < 0.0001$), and after six months (T6) IPAP was 23 (24.5–21.5)cmH2O ($p < 0.0002$) and TV 591.5 (717-484) ml ($p < 0.0001$). The EPAP was maintained as 5cmH_2O. The SaO_2 without AVAPS was 96 (96–95.5)% and with AVAPS 97 (97–95)%. Adjustment of IPAP was needed at T3 and T6.

Discussion and conclusions: Consecutive evaluations through BIPAP® downloads are extremely important to NPPV sucess in ALS patients. In this study we observed

an increase of TV when we compared BIPAP® with and without AVAPS. At T3 and T6 a readjustment in IPAP was necessary to maintain the TV established on ST, probably due to the decreased thoracic complacence. The AVAPS function is a gold standard to find the ideal IPAP for MND/ALS patients.

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P17 SURVIVAL OF AN ALS POPULATION NON-COMPLIANT TO NIV: IS THERE ANY RELATIONSHIP TO PARAMETERS SETTING?

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Background: ALS survival depends on Non-invasive ventilation (NIV) and on its compliance. In a related study (see abstract C95), we identified women with bulbar-onset, late NIV intervention and low respiratory muscle strength, particularly prone to be non-compliant to NIV. Whether these conditions are related to NIV parameters settings, it is not known.

Objective: To correlate NIV parameters setting to survival and compliance to NIV.

Methods: We followed-up prospectively 23 consecutive ALS patients adapted to one particular type of ventilator (BIPAP ®) and recorded initial and sequential ventilator parameters settings and compliance characteristics of the compliant (group 2) and non compliant patients (group1). For comparison of the groups we used t-test, Chi^2-test, univariate and multiple regression.

Results: Group 1 included 9 pts, 7F (3 Spinal and 4 Bulbar) and 2M (1Spinal and 1Bulbar) mean age (mean $\pm$ SD) 61 ± 11 years with an overall compliance lower than 70%. Group 2, included 14 pts 5 F (2Bulbar and 3 Spinal) and 9 M (7 Spinal and 2 Bulbar) mean age $\pm$ SD (55 ± 12) yrs and compliance over 70%. At admission gender was the only significant difference (p=0,01). Gender differences affected PImax, P0.1 and Amplitude (0,05; 0,05; 0,01).). Initial parameters settings were identical in the two groups showing similarity of procedures. When considering parameters settings and compliance recordings and type of disease onset in the two groups, back-up rate (BPM) and the number of apnea were different (p=0,03; 0,05), both being higher in spinal forms. In addition they also showed a trend towards a lower % of spontaneous respiratory cycling. Time to compliance and the number of parameters changing settings were also different, being larger in Group 1. Tidal volume and other compliance parameters were non-significant. Sequential changes in parameters showed differences in IPAP, maximal inspiratory time, BPM and % of spontaneous respiratory cycling and number of hours of use respectively (p=0,07; 0,03;0,03; 0,07; 0,03). In univariate regression compliance was related to survival as expected and to IPAP, BPM and inspiratory peak flow. However in a multivariate model only IPAP was a significant independent variable. When adjusted for disease onset and gender the significant independent variables were BPM, % of spontaneous respiratory cycling and the volume of leaks (p=0,02;0,04; 0,05).

Discussion: Despite similar procedures, these results show that different actions must take place in Bulbar women, in order to reduce the % of spontaneous respiratory cycling by increasing IPAP and back-up rate of the ventilators.

P18 ADHERENCE TO NIPPV IN ALS PATIENTS WITH MILD AND MODERATE RESPIRATORY IMPAIRMENT: PRELIMINARY RESULTS OF THE PILOT NUTRITION/NIPPV STUDY

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Background: Recent studies have indicated that Noninvasive ventilation (NIPPV) improves quality of life and significantly prolongs survival in Amyotrophic Lateral Sclerosis. An evidence-based review of the subject indicated that most ALS patients develop symptomatic hypoventilation by the time FVC falls below 50%. The authors provided a management algorithm for NIPPV based on symptoms and FVC recommending that NIPPV be initiated when FVC falls to 50% or the patient is dyspneic. However, the optimal timing of NIPPV remains undefined and factors contributing to compliance remain largely unexplored. As part of a multicenter pilot study of NIPPV and nutrition in ALS (PEGNIV), one of the issues under investigation is whether a large Phase III trial comparing outcomes between earlier initiation of NIPPV (at 80% FVC) with initiation at the present guidelines (50%VC) would be feasible. A major concern is whether asymptomatic patients will accept and use NIPPV.

Objectives: To determine if ALS subjects who are started on NIPPV before respiratory symptoms or decreased vital capacity occur will be adherent with use of NIPPV

Methods: Subjects who are enrolled in the NIPPV arm of the study are stratified into two groups based on FVC at entry. Group 1 includes participants who have an FVC between 80 and 95%. Group 2 subjects have an FVC between 50 and 80%. Group 1 patients are offered NIPPV (Respironics, Synchrony) at 80% FVC while Group 2 subjects are initiated at 50% FVC. Participants received a standardized educational program on NIPPV in ALS. In addition, frequent home visits by respiratory therapists occurred at the start up of the NIPPV. NIPPV use was downloaded directly from the Synchrony including the average daily use and the percentage of days with usage greater than four hours.

Results: To date there are 31 patients enrolled in Group 1 with a median age of 59, 68% are male and 32% female. There are 23 patients in Group 2 with a median age of 60 years, 61% male and 39% female. In Group 1, 25 patients have been offered NIPPV. Of these 25 patients, 4 patients did not accept NIPPV. Adherence data is available on 13 patients and indicates that patients used the NIPPV for more than four hours on 82.2% of days with an average daily use of 4 hours and 58 minutes. In Group 2, 19 patients have been offered NIPPV and 2 rejected. Adherence data was available from 10 patients and indicates that the subjects used the NIPPV for more than four hours on 96.5% of days with an average daily use of 7 hours and 4 minutes.

Discussion and conclusions: Preliminary results indicate that subjects with little or no respiratory impairment will accept and use NIPPV for greater than four hours daily. This level of acceptability will enable further studies to be designed in order to examine clinical efficacy of early vs late initiation of NIPPV. The high level of adherence in both groups is likely related to several factors including motivation, education, and the frequency of respiratory therapist visits.

P19 DECISION MAKING ON MECHANICAL VENTILATION BY PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Japan had about 7300 patients with amyotrophic lateral sclerosis (ALS) in 2005 and a relatively high rate of ventilator use compared to other countries. Ventilation increases the burden on caregivers, making it difficult for patients to decide.

Objective: To understand the factors related to the decision to use ventilation.

Methods: This was a qualitative, descriptive study carried out from May to September, 2005. The participants were 5 patients with ALS (3 males, 2 females) who agreed to be interviewed and who met the following criteria: 1) knew of their diagnosis; 2) received an explanation of ventilation; 3) not currently on ventilation; 4) capable of conveying their thoughts.
Information was collected during a semi-structured interview on their thoughts at diagnosis, on changes in daily life as symptoms progressed, and on using ventilation. The interview was transcribed and the data coded and categorized.

Results: Four categories, which interacted with each other, were extracted as factors related to decision making: value attached to life, image of life after going on ventilation, opinions of others, and preconditions. Patients with ALS seemed to create an image of life after ventilation, organizing their thoughts about preconditions. Preconditions included living autonomously, not increasing the burden of caretaking such that it would change the lives of caregivers, keeping a line of communication, ability to go out, small economic burden, and ability to go off ventilation if the patient so desired.

Discussion and conclusion: We found that some patients with ALS make decisions about using ventilation by creating an image of life on ventilation using new information made available to them from specialists and other patients and their caregivers. The more detailed the image, the more they were able to compare the way they want to live with the possibilities of being on or off ventilation. In other cases, the uncertainty of the progress of the disease led to an avoidance of people, interfering with obtaining information.
Patients with ALS said the period after they learned of their diagnosis was the most painful. It is thus important to provide emotional support to patients during this period, when anxiety about the future is strong. Some patients talked openly with their families, but others found it difficult.
In conclusion, in order to make a decision about using ventilation that satisfies both patient and family, it is important for them, with the support of specialists, to fully discuss the decision on ventilator use.

P20 ARTIFICIAL VENTILATORY MANAGEMENT IN LONG-TERM HOSPITALIZED PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AT A JAPANESE HOSPITAL FOR CHRONIC NEUROMUSCULAR DISORDERS

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Background: Artificial ventilatory support has been an important procedure in the management of patients with amyotrophic lateral sclerosis (ALS). The requirement for radical essential treatment in hospital notwithstanding, the

ideal situation is for people with ALS to live with a ventilator at home. However, a significant number of patients with ALS have to be kept in hospital in Japan.

Objectives: The aim of this study is to clarify the actual condition of artificial ventilatory management of patients with ALS who have been in a hospital for chronic neuromuscular disorders for a long period.

Methods: Patients with ALS who had been under artificial ventilatory management in our hospital were retrospectively investigated. Our hospital specialises in caring for patients with chronic neuromuscular disorders, and 130 of 320 sickbeds are prepared for them.

Results: Forty-two inpatients were identified who had been in our hospital long term during a period from 1998 to 2006. Thirty-three patients had undergone artificial ventilatory support. Five patients died without ventilatory support. Two patients left our hospital to live at home. Two were transferred to other hospitals. Twenty-eight of 33 patients had ventilatory management with tracheotomy (TIPPV). Thirteen patients had non-invasive ventilatory management (NIPPV). Eight of 13 patients had to shift to TIPPV from NIPPV and 2 of 13 patients were dead. The mean duration to shift from NIPPV to TIPPV or death was 14 months. Nineteen of 28 TIPPV patients were still alive, and the mean duration of TIPPV management was 43 months. Nine of 28 TIPPV patients were dead, and the mean duration under TIPPV to death was 31 months. The most common cause of death was aggravation of respiratory infection, followed by sudden death, renal failure or malignant tumor. There was a tendency that patients who had adopted artificial ventilatory management earlier with good physical condition were alive longer than those in whom artificial ventilatory support had been initiated urgently during illness.

Conclusion: The experience of artificial ventilatory management in ALS at a Japanese hospital for patients with chronic neuromuscular disorders was summarized. Patients with ALS under TIPPV management who had accepted when in good condition could survive over 40 months or more whereas there might be limits of NIPPV support in ALS.

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THEME 2 RESEARCH TO IMPROVE STANDARDS OF CARE

P21 CHANGING PERSPECTIVES IN THE TERMINAL PHASE? A COMPARISON IN ALS AND CANCER PATIENTS

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Background: ALS patients are often perceived as positive minded and friendly patients. Data from our group confirms that ALS patients have a positive shift in the perception of social and emotional information (1, 2).

Objectives: To test whether the positive shift in the perception of ALS patients and the perception of ALS patients by others is a disease specific phenomenon or rather a consequence of the changed perspective in the terminal phase of life.

Methods: Socio-emotional processing (facial expressions of emotions test, FEEST), emotional and psychological disposition (Beck's depression inventory, BDI; ALS depression inventory, ADI-12; Freiburger Persönlichkeitsinventar, FPI; schedule for the evaluation of individual quality of life, SEIQoL) and assessment of the patient by their primary caregiver (coping, personality factors) were tested in 30 ALS patients and compared to the data of 30 cancer patients with palliative treatment and 30 healthy controls.

Results: Both patient groups presented an impaired ability to recognize emotional expressions in faces compared to healthy controls. ALS patients showed a stronger impairment in recognizing negative (anger, sadness, fear) facial expressions compared to cancer patients and presented a better performance in recognizing positive (happiness) or neutral (surprise) emotional expressions in faces. Concerning quality of life both patient groups had a similarly good quality of life. For ALS patients social factors (family, friends) had a significantly higher impact on their quality of life. The performance of recognition of negative emotional expressions in faces strongly correlated with the social factor in QoL.

Discussion and conclusion: The diagnosis seems to have an impact on the way a patient perceives the socio-emotional world around him. Both patient groups have a poor prognosis in common (both receive palliative treatment) and both present impairments in the recognition of faces. However, in cancer patients the impairments in face recognition abilities seem to be a more general phenomenon (probably a draw-back from the external to the internal world). ALS patients present a positive shift for their emotion recognition ability in faces meaning that the impairments seem to be restricted to negative emotional expressions. This positive shift seems to be disease specific in ALS patients. Since recognition abilities of negative facial expressions correlated with the significance of social factors in quality of life, the significantly higher satisfaction with the social surrounding in ALS patients compared to cancer patients might have an influence on emotional and social perception in ALS patients.

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P22 THE WISH TO HASTEN DEATH AMONG ALS PATIENTS IN A PALLIATIVE CARE PROGRAM

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Background: Amyotrophic lateral sclerosis (ALS) is a major challenge to palliative care, particularly as the characterics of the disease may provoke patients' wishes to hasten death.

Objectives: The study investigates the prevalence and determinants of the wish to hasten death in ALS patients and the opinions of their caregivers.

Methods: The semi-quantitative questionnaire study included patients and their primary caregivers enrolled in an outpatient ALS palliative care program.

Results: The study comprised a sample of 30 patient-caregiver-pairs. 31% of patients expressed the desire to hasten death. Suicidal ideation was admitted by 50%, 24% had planned and 6% actually tried suicide. 44% of patients could imagine asking their doctor for physician-assisted suicide or euthanasia. The desire to hasten death correlated significantly with loneliness and both the depression and anxiety subscales of the Hospital Anxiety and Depression Scale, but not with religiosity as measured by the Idler Index of Religiosity. Only 11% of caregivers said their relatives communicated with them about hastening death. 25% and 20% of caregivers could imagine assisting in suicide or performing euthanasia, respectively.

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Conclusions: The wish to hasten death is common among ALS patients in a palliative care setting. Its correlations with loneliness, anxiety and depression pose challenges to palliative care. Physicians and caregivers should address this issue more openly.

P23 ADVANCE CARE DIRECTIVES IN ALS: WHEN IS THE RIGHT TIME TO TALK?

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Background: Amyotrophic Lateral Sclerosis (ALS) is a devastating, progressive motor neuron disease, which is terminal in most cases. A distinguishing feature in the management of patients with ALS is an individualized advance care plan addressing specific decisions regarding end-of-life care. As the disease progresses, patients often need to decide whether they will receive invasive ventilation to prolong survival. It is generally accepted that these advance care directives should be discussed with all patients to respect their autonomy, however, the optimal timing for discussion regarding end-of-life care is unclear. While end-of-life care discussions at an early phase of the disease may be helpful for some patients to decide on advance directives with family members, it may provoke premature anxiety and stress. Alternately, the need for invasive ventilation or death may precede end-of-life discussions if the clinical team waits until the advanced stages of the disease.

Objectives: The aim of this study was to determine patient/family member preferences regarding the optimal timing for discussions pertaining to end-of-life care and advance directives. We also examined which factors are associated with the timing preference for end-of-life discussions.

Methods: Thirty patients with clinically probable or definite ALS were surveyed regarding the preferred timing of end-of-life care discussions. The primary caregivers of 30 deceased ALS patients were also surveyed to determine when end-of-life discussions occurred and inquired about their preferences. Basic demographic and socioeconomic data was also collected.

Results: The majority of ALS patients (approximately 75%) preferred early education and discussions (within 3–5 months following diagnosis) regarding end-of-life care and decisions despite the additional stress that was provoked. Age, increased years of education and higher income were strongly associated with a preference for earlier timing for discussion. This study also showed that primary caregivers with strong spiritual and religious beliefs preferred early end of life care discussions. Data from other predictor variables will also be presented.

Conclusions: The majority of patients with ALS preferred to discuss end-of-life care at an early phase of the disease, despite the additional anxiety that was provoked by these discussions. Older patients, patients with a higher level of education and income and primary caregivers with strong spiritual and religious beliefs tended to favour earlier discussions. Other factors that predict patient/family preferences for early vs. late end-of-life care discussions will also be presented to assist clinical teams in choosing the optimal timing for an individual patient.

P24 PERSPECTIVES ON FACTORS AFFECTING ACTIVITIES AND PARTICIPATION – IMPLICATIONS FOR MULTIDISCIPLINARY CARE FOR THE AGING POLIO SURVIVOR IN A CANADIAN SETTING

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Background: Little is understood about factors that impact the lives of persons living with poliomyelitis, and the benefits that a multidisciplinary approach to care may have, with respect to life activities.

Objective: Identify important factors affecting the activities and participation of persons living with poliomyelitis, and provide recommendations for multidisciplinary involvement as persons with poliomyelitis age.

Methods: Four cases of participants living with poliomyelitis are presented. In-depth semi-structured interviews guided by all categories of two outcome measures were conducted. The Barthel Index measuring activities of daily living, and the Participation Scale measuring participation were used. Mixed methods were used, employing qualitative and quantitative analysis of the data. Audiorecordings of all interviews were transcribed verbatim. Data were entered into NVIVO 7,^{TM} codified, and analyzed according to themes. Self-reported ratings of factors identified as affecting each outcome measure category were analyzed quantitatively. Factors that were reported by all four participants were identified, along with any expertise required to facilitate such factors.

Results: Participants identified progressive changes in their abilities, and the need for ongoing adaptations as they age. Factors having highest collective rankings affecting the Barthel Index (1) categories, were identified as mobility aids, adaptive modifications, and adaptive equipment, in order of importance. Factors affecting categories of the Participation Scale (2) in order of collective importance, were identified as personal attributes and circumstances, impairments, accessibility, family and friends, mobility devices, transportation, adaptive strategies, policies and systemic factors, and income. Personal perspectives on the importance of factors are shared. All four participants listed these contributory factors to life activities: personal attributes and circumstances, family and friends, services, income, and impairments.

Conclusions: As persons living with poliomyelitis age, they report progressive impairments, that require adaptations in order to maximize activities of daily living and participation. A longitudinal multidisciplinary approach, including involvement of social workers, occupational therapists, physiotherapists, physicians is recommended. In the Canadian health care delivery system, physicians are often gatekeepers, and are well positioned to advocate for, and facilitate such coordination of care.

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P25 PALLIATIVE CARE AND CIRCUMSTANCES OF DEATH IN PATIENTS WITH ALS USING NON-INVASIVE VENTILATION IN GERMANY – A SURVEY OF PRIMARY FAMILY CAREGIVERS

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Background: Non-invasive ventilation (NIV) is known to improve quality of life and to prolong survival in amyotrophic lateral sclerosis (ALS). Little is known about circumstances of death in ventilated ALS patients. In the light of the debate on legalizing euthanasia it is important to provide empirical data about dying and death in these patients.

Methods: In a semi-structured interview 29 family caregivers of decedent ALS patients were asked about their own and the patient's attitude toward NIV, physician-assisted suicide (PAS) and euthanasia, circumstances of death, fear of death and preoccupation with death.

Results: Of five patients nonrecurring suicidal thoughts were reported; three patients and seven relatives thought about PAS. In none of the investigated cases indices of suicide, assisted suicide or euthanasia existed retrospectively. In 55.1% the issue of death was discussed within the family or partnership; only one patient expressed mortal fear but 17.2 % were afraid of choking. From the caregivers' view 75.9% were classified religious. Seventeen caregivers described the patient's death as "peaceful" while choking was reported in 6 bulbar patients. In final stages the general practitioner (GP) was involved in 10 patients' care. Palliative medication including sedatives and opiates was administered in eight patients by the GP. 62.1% died at home, 37.9% in hospital.

Conclusion: Suicidal ideas or the wish for PAS or euthanasia seem to be low compared to previous investigations in other countries. Besides the legal situation (euthanasia is illegal), a bias due to selection of NIV-patients and a high percentage of religious patients might account for this. Though most patient died peacefully, the high rate of choking in bulbar patients and the low rate of GPs involved in terminal care requires clinical attention.

P26 EYE SWITCH COMPUTER CONTROL FOR COMMUNICATION RESTORATION FOR THOSE WITH MND

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Background: The MND Association of Taiwan estimates there to be between 800 and 1000 people with MND in the country. Of the approximately 250 registered with the association, over half have been identified as requiring a communication device, especially those whose only residual movement is eye movement. However only a minimal number have actually received a communication aid, something the association seeks to improve. With limited government funding, new creative low cost communication solutions are needed.

Objectives: To find a low cost PC based eye control solution to resolve the communication difficulties of those with MND in Taiwan.

Methods: Eye tracker systems for computer control all suffer from exceptionally high costs especially for the more mature and stable systems. This financial constraint limits their use to all but a few users in Taiwan. Rather than use the complex 2-D positioning techniques of mouse trackers, as an alternative, the concept here was to use the inherent Electro-Oculogram (EOG) signal from the eyes to generate a single switch output signal. The EOG signal is an electrical signal of very low amplitude, which varies in vector position if the eyeball is rotated from its central resting position. Using standard skin electrodes, if this signal can be detected, amplified and converted to a computer USB input signal, it can then be used to control a scanning type screen keyboard, which can be used for communication purposes.

Results: Results from a single user with MND has surpassed expectations and found the user able to control the scanning keyboard after only a short training period. This has provided the motivation to move forward and develop the experimental device into a commercial low cost product. The device will also include a fully integrated remote control nurse call function.

Discussion and conclusions: The concept of computer control using an EOG signal instead of the usual infra-red eye tracking methods has proven to be successful. With a suitable scanning screen keyboard communication can be restored at relatively low cost thus increasing availability. The feasibility of using the EOG switch for internet access in addition to scanning keyboard control is now under investigation and offers an additional application for this control method.

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P27 GENERAL ANAESTHESIA IN PATIENTS WITH ALS/MND CAN BE SAFE: LESSONS LEARNED FROM THE TECHNIQUES USED IN THE DIAPHRAGM PACING STIMULATION (DPS) TRIAL

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Background: There is a paucity of literature and no standard for the conduct of general anaesthesia in patients with MND/ALS. Management has historically paralleled recommendations of non-ALS patients undergoing similar procedures but this has lead to hemodynamic and neuromuscular complications in some patients. The current trial of the laparoscopic diaphragm pacing stimulation system (DPS) necessitated general anaesthesia without compromise of diaphragmatic or neuromuscular function either intraoperatively or postoperatively. This report describes the anaesthesia management in 18 ALS patients undergoing DPS implantation as an outpatient laparoscopic surgery.

Objectives: Identify the optimum techniques of applying general anaesthesia to patients with ALS/MND.

Methods: Our overall strategy was to use rapid reversible short acting analgesic and amnestic agents without neuromuscular relaxants to minimize side effects in patient with neuromuscular diseases. The following regimen was used in all patients: midazolam (anxiolytic and decreases intraoperative muscle spasms); remifentanil (intravenous ultrashort acting narcotic with rapid on and off capabilities, used for induction and maintenance of anaesthesia. This agent, being a potent narcotic, depresses the respiratory drive which facilitates our mapping technique of diaphragm pacing because during the operation the patient will not be trying to breathe which may interfere with surgery. Because of its ultra short action, the patient resumes their normal respiration minutes after discontinuation of the drug.); sevoflurane (inhalational amnestic agent with low lipid solubility allowing rapid on and off) and propofol (a short acting intravenous amnestic agent with rapid on/off capabilities). At the end of each procedure the DPS system is also utilized to increase the respiratory system compliance by decreasing posterior lobe atelectasis. If a patient was on non-invasive positive pressure ventilation pre-operatively they are placed on it in the recovery room. We can now also measure continuous diaphragm electromyography (EMG) via the implanted electrodes to assess diaphragm function immediately post-operatively.

Results: To date we have used this in 17 patients and have found no adverse problems with general anaesthesia. The average predicted forced vital capacity(FVC) at surgery was 52% with 5 patients below 50%. All patients were extubated uneventfully at the completion of surgery. No reintubations were needed. All patients were discharged from the hospital with no respiratory problems.

Conclusions: Patients with ALS may require surgical procedures during the course of their disease (for example appendicitis, cholecystitis) and an understanding that general anaesthesia can be safely given to patients will increase the quality of their life. General anaesthesia consisting of remifentanil, sevoflurane and propafol was effective in facilitating neuromuscular evaluation and laparoscopic surgery in ALS patients without adverse perioperative effects. This strategy may be useful more widely in surgery on patients with ALS. Consideration should be given for implantation of the DPS system at the time of surgery for peri-operative management and increasing peri-operative respiratory compliance.

P28 BAD NEWS COMMUNICATION AND INFORMATION SEEKING BEHAVIOUR IN ALS PATIENTS AND CAREGIVERS

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Background: Bad news communication is a complex process, in particular in chronic and severe disorders such as ALS. However no study on the perspective of news communication in ALS patients and caregivers has been performed.

Aim: To evaluate the expectation of and satisfaction with bad news communication and use of other sources of information in a series of ALS patients (PTs) and caregivers (CGs).

Methods: A series of 60 consecutive ALS patients and informal caregivers were interviewed using a structured questionnaire concerning several aspects of the bad news communication. The questionnaire was administered separately to PTs and CGs. Single and multiple correlations were performed between the various items of the interview and clinical and demographic factors.

Results: The PTs were 35 men and 25 women, with a men age of 63.4 (SD 9.5) years and a mean duration of ALS of 1.5 (SD 0.8) years. The CGs included 33 spouses, 19 sons/daughters, 8 other relatives or friends. Compared to CGs, PTs were more satisfied with communication ($p=0.05$) and felt that the neurologist had adequately understood their inner feelings ($p=0.0006$). The most important aspects to be communicated were current research about ALS, therapies that can slow disease progression, and ALS outcome for both

PTs and CGs. Significantly more CGs than PTs ($p=0.0008$) searched for additional information. The most frequently searched for alternative sources of information were internet and patients' associations (PTs), and internet and newspapers (CGs). However, for PTs the most reliable and comprehensive sources were medical meetings, television, and patients' associations, whereas for CGs they were medical meetings, patients associations, and friends. PTs felt that the caring neurologist revealed the most relevant information and that they knew almost everything about the disorder. PTs gender and level of education, as well as ALS clinical type and duration did not influence any of the examined items, whereas an older age was related to a higher satisfaction for the communication ($p=0.05$) and to a greater tendency to feel that the caring physician had revealed all relevant information ($p=0.01$).

Conclusion: PTs and CGs were satisfied by the bad news communication process, but they understandably felt discouraged. The caring neurologist should better attune the content of bad news communication to PTs' and CGs' preferences. Health professionals should be aware that ALS PTs and CGs often use the internet and other media to obtain medical information and should help them to better sort out and interpret the news they find.

P29 INFORMAL CAREGIVING: ITS IMPACT ON ALS PATIENTS' AND THEIR CAREGIVERS' QUALITY OF LIFE

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Background: Most people with ALS (PALS) are able to access their country's disability programs. Nevertheless, the burden of care (BOC) for informal caregivers (ICs) increases dramatically as disease progresses. Little to no provision of support for ICs from disability programs is available, which can increase BOC and therefore lead to decreased quality of life (QOL), higher morbidity and mortality, and loss of employment among ICs. These factors can lead to institutionalization of PALS, which is costly. These increasing costs may lead to less support for PALS from disabled programs attempting to control costs, exacerbating the problem.

Objectives: Determine from the literature if there is a lack of informal caregiving (IC) resources for ICs of PALS that leads to increased BOC and causes decreased QOL among PALS/ICs. Organize the determinants of QOL using Green's PRECEDE-PROCEED model (1) through a meta-analysis of the ALS caregiving literature for disabled PALS and their ICs.

Methods: A meta-analysis of the ALS/MND literature was conducted concerning ''quality of life'' and ''caregiving''. Inclusion and exclusion criteria were using the major health, public policy, and multidisciplinary databases. Results include a total of 51 reviewed articles organized using Green's PRECEDE-PROCEED model.

Results: QOL of PALS/ICs are determined by a variety of factors, organized here into distal and proximal factors using Green's model: Examples of Distal Determinants: Predisposing factors that improve/worsen QOL include purpose in life and sense of spirituality; Reinforcing factors that improve/worsen QOL include use of a multidisciplinary clinic and lack of mental health support; Enabling factors that improve/worsen QOL include presence of disability programs and lack of payment for ICs. Examples of Proximal Determinants: Environmental indicators that improve/worsen QOL include less severity of disease, higher socio-economic status, and worsening disability; Behavioral indicators that improve/worsen QOL include attending support groups, history of depression, and PALS with cognitive difficulties.

Discussion and conclusions: According to the literature there is a lack of IC resources and providing more resources for IC would decrease the BOC of ICs and increase the QOL of PALS/ICs. More research needs to be conducted to determine the impact and magnitude of these determinants on QOL. The literature describes many of the proximal determinants that improve and worsen QOL in PALS/ICs. These research-based determinants should be organized, discussed, and formally put into practice in the ALS community to help improve the QOL of PALS/ICs. The literature shows major gaps in the distal determinants of QOL and this area needs more research because they affect the provision of care on national levels and determine access to resources from insurance systems and health care providers, as well as shape societal values concerning disability and support for those with ALS.

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P30 ALS CAREGIVER CONFERENCES: OUR EXPERIENCE

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Background: Given the many challenges faced by ALS caregivers (CGs), we organized two half day CG conferences that included vendor displays and educational talks. While CGs frequently accompany the patient to the clinic, the focus is on patient care, and CGs' needs are not always addressed. Because of this, as well as our concern that CGs may be less likely to ask certain questions with the patient present, the conferences were for CGs only, patients did not attend.

Objective: To describe the content of the conferences, and how our experience with the first influenced the development of the second.

Methods: The first conference (CGC1) was structured as follows: during 2 blocks of time 2 breakout sessions were held with registrants selecting the talk they wished to hear beforehand. For the second conference (CGC2), we had 3 sequential talks, so all attendees heard all speakers. At both, the keynote address was given at lunch and all attended. Keynote speakers were from out of state and had spoken before at ALS related events, all other speakers were local. We surveyed attendees at both conferences.

Results: CGC1 was held in October 2005 and CGC2 in April 2007. There was no charge to attend either conference, funding was through vendor booth fees and our local ALS/Motor Neuron Disease Fund. Forty-five CGs attended CGC1 and 26 CGC2. Breakout session topics at CGC1 were maintaining nutrition or hospice and advance directives, and respiratory management or accessing services. The keynote address was entitled: Rest, Relax, Renew. Topics covered at CGC2 were caring for the caregiver, adaptive equipment, and hospice. The first 2 topics were chosen based upon suggestions from CGC1 participants. The keynote address was entitled: Live with Hope, not Fear. Evaluation response rate was 67% from CGC1 and 73% from CGC2. Using a 5 point scale with 5 strongly agree and 1 strongly disagree, the mean score for ''The conference met my expectations'' was 4.7 for CCG1 and 4.9 for CGC2. For individual talks, scores to the question ''The talk was informative'' ranged from 4.5–4.9 for CGC1 and 4.7 to 4.9 for CGC2. Keynote addresses were particularly well received, both achieving mean scores of 4.9. Ninety per cent of the attendees from CGC1 and 100% from CGC2 felt the length was just right. Since 52% of respondents from CGC1 felt that sequential talks would be better, we changed the structure of CGC2. Of the 6 who attended both conferences, 100% felt the sequential format was preferable to the breakout sessions.

Discussion and conclusions: Half day ALS caregiver conferences are well received by participants. Though attendees overwhelmingly stated they would be interested in attending another conference, repeaters were in the minority. This may be useful to keep in mind when determining the interval between events.

P31 IMPLEMENTATION AND EVALUATION OF A SOCIAL SUPPORT PROGRAM FOR ALS CAREGIVERS USING PEER MENTORS

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Background: Caregiver burden is a major issue in ALS, especially when social supports are lacking. Yet, as the illness progresses, caregivers do acquire much knowledge about different facets of care for the ALS patient. A mentoring program, wherein former ALS caregivers mentor current ALS caregivers, might facilitate the ability of current caregivers to cope and reduce caregiver burden.

Objectives: We wished to implement and evaluate a social support program for current ALS caregivers, using trained former ALS caregivers to help new caregivers establish or extend their support network, develop or expand their knowledge and understanding of ALS and community resources, and provide caregivers with emotional support.

Methods: We recruited 12 former caregivers to ALS patients who had received care through the McMaster ALS Clinic. These mentors were trained and instructed to provide informational and emotional support to a current caregiver of a patient with ALS diagnosed through the Clinic within the previous 12 months. The 12 current caregivers were assessed at onset, and prospectively throughout a 12 month period, using several scales: the Inventory of Social Supportive Behaviours, the Caregiver Self-Efficacy Scale and General Self-Efficacy Scale, and a satisfaction scale. To assess the impact of disease severity, we included the ALSFRS. Mentors completed a tracking sheet after each visit outlining the issues/topics discussed, length of the conversation, and amount of time spent providing emotional/information support. The mentors met as a group once a month to debrief. Open-ended questions were included to give both the mentors and caregivers an opportunity to share their observations about the program. The study was approved by the McMaster University Research Ethics Board.

Results: Two matches were terminated shortly into the program. One patient died; one current caregiver declined to participate. However, at termination, all remaining current caregivers stated that they benefited from the program and 8 (of 10) wished to continue past the study period. Surprisingly, all 12 mentors felt they benefited from the program, and all wished to continue with current or new caregivers. The benefits to the current caregivers were not captured in the scales we used.

Discussion: A support program pairing former caregivers with current caregivers was felt to be useful by all current caregivers. The scales we employed to measure outcomes were less informative than simple satisfaction scales, and may not reflect the true utility of the program. All former caregivers felt the program was beneficial, and we speculate, without proof, that the program helped them with the grieving process.

Acknowledgement: The authors are grateful for the support of the Douglas family, and of Mr David Hunt, that allowed this study to occur, and for the support of the ALS Society of Ontario.

P32 SYNCHRONIZED IDENTIFICATION IN FAMILY CAREGIVERS (WIVES) OF PATIENTS WITH ALS

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Background: For patients with ALS, each day brings new losses, characterized by increasing physical dependence on

others. In Japan, 26.8% of ALS patients use ventilation, but 24-hour home care for patients with ALS is not covered yet. Therefore, one of the challenging things is how to reduce the burden of family caregivers.

Objectives: The purpose of this study is to clarify what happens in the lives of patients with ALS and their wives by using the narratives of the wives, focusing on the unique relationship that they establish in the long-term struggle against the disease and through caring.

Methods: The data collection was as follows: 1) the participatory observation; 2) the unstructured interview; 3) the semi-structured interview with wives who were caring for patients or were bereaved. Participants in this study were 12 wives. The data were analyzed in a qualitative and descriptive way.

Results: The wives developed extreme alertness until they acquired the ''synchronized identification'' with their patients about their actions, body and mind. They then are afraid that they will be intruded upon by someone else who is going to help them in their house, so that they tend to exclude any help. They become ''Selves being only in the relationship with their patients'', although there are some wives who try to restore their own lives by restarting their hobby or participating in the meeting of patients. To survive the extremity of ALS, the wives display dissociation such as difficulties of recollection and fragmentation of memory and narrative. Some wives felt guilty for not being able to persuade their patients to use the respirator to live or not knowing the patients' wishes to die in peace, though several years have passed since their patients died.

Conclusions: The family caregivers appear to not need any support from the experts but actually this is not the case. They need help but they don't know where to go and ask for support. On the whole, they can take care of their patients just like professionals, so they tend to think that the expert team is not up to themselves. That's the problem. The wives tend to be confined to themselves. It causes them mental fatigue. They also become seriously sick from the cumulative exhaustion. This study provides a new perspective in understanding their helplessness. Respite care, for example, can be one good way of enabling wives to take a rest whenever they want without hurting their feelings.

P33 QUALITY OF LIFE IN PATIENTS WITH ALS AND THEIR INTIMATE

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Background: A progressive debilitating disease causes a change in the quality of life (QoL) both in patients and their intimate. Support for patients and intimates should be given to all in need according to the needs identified at different points of the course of the disease.

Objective: The aim of this study was to study the QoL in patients with ALS and their intimate in relation to physical function throughout the course of the disease to identify the life events of importance for QoL.

Methods: All patients had definite or probable ALS. Forty-four patients and 37 intimates participated. The ratings were performed by SEIQol, SF-36, HAD and visual analogue scale (VAS; general, physical and mental well-being). Patients and intimates also made estimates of their counter-part in their relations at intervals of 4.5 to 6 months during the course of the disease. Function and state were assessed by Norris and ALSFRS-R scales.

Results: The study showed that intimates had a higher rating of anxiety and patients had a higher rating of depression by the HAD scale. Female intimates had a higher score of anxiety than all other groups over time. There was a good correlation between ALSFRS-R and Norris scales over the course of the disease.
The VAS ratings showed no major change from first to later ratings. The intimates rated their mental well-being lower than the patients and the patients rated their physical well-being lower than for intimates. The intimates made an underestimate of the well-being of the patient on all VAS parameters while patients estimated the well-being of their intimate at the same level as the self-estimate by their intimate.

Discussion: Few studies on the well-being and QoL of patients focus also on the well-being of the intimate and even fewer studies follow the changes over the time course of the disease. We have found that there is only a limited understanding between the patient and their intimate with respect to the feelings and strain that the other person experiences. If more knowledge is acquired in this respect, we will be able to support patients and their intimates better at a time critical for their QoL.

P34 QUALITY OF LIFE OF ALS PATIENTS IN SERBIA

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Background: Amyotrophic lateral sclerosis (ALS) has a severe impact on patients' quality of life. Measuring life quality in ALS patients helps us to assess their health status. Such data are also useful in sample size calculations for future treatment trials in which quality of life is a primary outcome variable.

Objectives: To assess quality of life of ALS patients by using Short Form-36 (SF36),a self administered generic measure of health-related quality of life.

Methods: Fifty one ALS patients (21 females and 30 males), with probable or definite ALS according to El Escorial criteria referred consecutively to the Institute of Neurology, Clinical Center of Serbia, Belgrade, over a 1-year period. Patients with dementia or who suffered from a serious disease other than ALS were excluded. MOS SF-36 and Functional Rating Scale (ALS-FRS) were applied in all cases.

Results: Forty ALS patients with bulbar onset and 11 with limb onset were studied. The mean age of all patients was 55.3 years (range, 34–71) and the mean duration of disease was 2.5 years (range 1–7). Total SF-36 score was 37.6. The lowest response rates were gained in the domains of Role-physical scale which evaluates the extent of physical capabilities (14.8) and emotional functioning (17.26).The highest response rate was gained in the domain of bodily pain, a scale that evaluates the perceived amount of pain experienced during the previous 4 weeks and the extent to which that pain interfered with normal work activities (60.8). Patients with limb onset of disease have significantly better social functioning in comparison to the patients with bulbar onset. Positive correlation was found between Physical Component Scale (PCS) score and ALSFRS score and negative correlation between PCS and the age of ALS patients and also between PCS score and the age at the onset of ALS.

Conclusions: This pilot study is the first step in determining the quality of life of our ALS patients and serves as a basis for further assessment of therapeutic procedures.
The results of this study showed that much has to be undertaken to help our ALS patients realize their capacities for coping with disability and to improve their quality of life.

P35 HOPE, QUALITY OF LIFE AND WELL-BEING IN PALS AND THEIR CAREGIVERS

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Background: Hope has been studied and quantified in consumerism, psychology, education, in clinical medicine, and care-giving. A three component Hope Scale has led to understanding that higher-hope patients may live longer and have improved quality of life in certain types of diseases and injuries. Cognitive interventions such as using hope enhancing counselling strategies have been demonstrated to increase hope in certain individuals. Quality of Life (QoL) is a reliable measurement currently used in ALS as a self report measure. Spiritual Index of Well-Being (WB) is a reliable indicator of 3 components of quality of life; well being, self efficacy and life schema. ALSFRS-R is a standard used to determine disease progression in ALS.

Objectives: Using the above noted measurements, we wanted to quantify and compare hope, QoL, and WB in ALS patients and their caregivers. In addition, we wanted to address in what manner hope might be encouraged in this patient/caregiver population in order to improve quality of life and, possibly, extension of life. The long range goal of this project is to use the data obtained to develop cognitive interventions that will improve all the variables examined.

Methods: We assessed hope, QoL and WB in 13 people with ALS and in their 13 caregivers. In addition, ALSFRS-R was assessed in patients. A demographics form asked age, gender, ethnicity, marital status, age at onset of symptoms, and employment questions.

Results: Patients report higher levels of Hope, WB and QoL than their caregivers. There were positive correlations between WB and Hope in ALS patients; however, QoL was not related to hope. ALSFRS-R was positively related to hope, but not to QoL or WB. None of the patients' scale scores were correlated with caregivers' scores. Eight caregivers worked outside the home an average of 40 hours per week in addition to providing care for the ALS patient.

Discussion: ALS patients report higher levels of hope, QoL and WB than their caregivers. This study shows that more support for the ALS caregiver is needed. Some support might include: learning hope enhancing strategies from counselling psychologists; support groups for ALS caregivers led by experienced counsellors skilled in hope enhancing strategies; recommending early and frequent respite for caregivers.

P36 COPING STRATEGIES AND DEPRESSION IN ALS AND MS

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Background: Few studies have analyzed the relationships between depression and neurodegenerative disorders while, conversely, this condition is frequently noted in the affected patients. Coping strategies (CS) may be different between different chronic disorders and a better understanding of specific adaptations in a particular disease could be helpful for patient management.

Objectives: To compare CS and quality of life (QOL) in multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) to determine 1) which CS could be more protective regarding depression ; 2) the profile of change in QOL in the course of these diseases.

Methods: There were 14 MS patients, 11 women and 3 men, aged from 32 to 75 yrs (mean 45.6 yrs) and 14 ALS patients, 3 women and 11 men, aged 32 to 78 yrs (mean 63 yrs). Patients with dementia were excluded. They answered the following questionnaires and tests: coping scale WCC from Lazarus, short version, Beck's scale for depression (BDI-II) and QOL evaluation with NADL.

Results: ALS had the largest impact on activities of daily living (ADL) and instrumental ADL: $p=0.0015$ and $p=0.0014$, respectively. There was, both in ALS and MS, a correlation between the severity of depression and CS focused on emotion ($r=0.557$, $p=0.0017$). But, CS were not different between ALS and MS patients. There was a reverse correlation in both diseases between depression and quality of social life ($r=-0.490$, $p=0.0073$). Depression score was higher in ALS but this did not reach statistical significance.

Conclusion: Severity of depression is similar between ALS and MS, both groups use similar CS. In ALS, loss of autonomy is the major reason for the decrease in QOL. On a daily basis, spouse and home carers are the most important persons for a patient. In helping him to be an actor of his life and death, they actively contribute to self estimation maintenance.

P37 PREVALENCE AND RELATIONSHIP OF FATIGUE AND DEPRESSION IN ALS PATIENTS

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Background: ALS patients report symptoms of both fatigue and depression. It is not clear to what extent they occur together, how each relates to ALS disease status, or how stable are their manifestations.

Objectives: This study was designed to assess symptoms of depression and fatigue, as well as data on ALS disease status, for patients attending an ALS interdisciplinary center for routine 3-month visits.

Methods: Patients were asked to complete two self-rating forms: the Fatigue Severity Scale (FSS) and the Patient Health Questionnaire (PHQ), which provides a quantitative measure of depressive severity and an approximate clinical diagnosis of depression. ALSFRS-R scores and FVC were also assessed.

Results: 185 patients completed the self-ratings once; of these, 52 completed them twice, and 63, 3 or more times. At baseline, mean ALSFRS-R score was 33 (SD=8.4) and FVC 74% (SD=22). Mean FSS score was 36 (SD=15), PHQ score 6 (SD=5) and the correlation between the two was +.53 ($p<.00$). Interpreting the self-report scales categorically, 69 patients (37%) had clinically significant fatigue (defined as 41+ on the FSS). Thirteen (7%) scored 15+ on the PHQ, indicating a possible depressive disorder. Twelve of the 13 ''depressed'' patients (92%) had clinically significant fatigue; however, 57 of the 69 patients (83%) in the clinically fatigued category were *not* depressed.

The 69 patients with high fatigue at baseline had significantly lower mean ALSFRS-R scores (30 vs. 35, $p=.001$) and lower mean FVC ratings (67% vs. 77%, $p<.05$), compared to those with lower fatigue; age did not differ. Comparing the patients with and without depression, only age differed (67 vs. 61 years, respectively, $p=.05$).

Of the 69 patients with high levels of fatigue at baseline, 33 were evaluated again 3 months later. Of the 33, 29 (88%) continued to have FSS scores 41+. Nine of the 33 high fatigue patients were evaluated on a third occasion (6 months after baseline) and all 9 (100%) continued to have FSS scores 41+.

Of the thirteen patients with PHQ scores 15+ at baseline, 7 were reassessed 3 months later. Five of those patients (71%) continued to have scores in the ''depressed'' range. Four of the seven were evaluated for a third time, 3 of whom (75%) continued to indicate depression.

Discussion and conclusions: Clinically significant fatigue was more prevalent than depression as measured by self-report in this sample of ALS patients, although the greater social acceptability of reporting fatigue might have contributed to this divergence. Among those assessed more than once, both depression and fatigue were persistent. Fatigue appears to be an independent symptom that warrants consideration when evaluating patient functioning.

P38 SUBJECTIVELY REPORTED SLEEP QUALITY, EXCESSIVE DAYTIME SOMNOLENCE AND FATIGUE IN ALS/MND PATIENTS.

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Background: Sleep disturbance is common in adults and the incidence of insomnia is estimated to be 27% to 35% in the healthy population (1) and about 66 % in patients with neurological diseases (2). Sleep complaints are also common in ALS/MND patients and it is well established that deteriorating respiratory function in ALS/MND patients is a major cause of sleep disturbance. However, beside breathing-related sleep disorder, there is no study that explores the quality of sleep in ALS/MND patients.

Objectives: 1) To determine the incidence of insomnia, excessive daytime sleepiness and fatigue in ALS/MND

patients. 2) To correlate the findings with disease onset, duration, markers of disease progression (FVC and ALSFRS-R score) and the use of non invasive ventilation.

Methods: Pittsburgh Sleep Quality Index (PSQI) questionnaire, Epworth Sleepiness Scale (ESS) questionnaire and Fatigue scale questionnaire were sent to 166 ALS/ MND patients. Patients' charts were subsequently reviewed to determine age, sex, time and site of disease onset, FVC and ALSFRS-R score and the use of NIV.

Results: Forty eight percent of patients responded to the questionnaires. Poor quality sleep as defined by PSQI > 5 was reported in 55% of patients, average or poor ESS was reported in 43% of patients. Fatigue was significant in 61% of patients. A moderate correlation (0.44) was found between poor sleep and fatigue. We were not able to find any correlation between PSQI, ESS, fatigue scale, disease duration, FVC, or ALSFRS-R scores. Bulbar presentation and use of NIV did not affect subjective sleep evaluation.

Conclusion: ALS/MND patients suffer poor sleep regardless of their FVC, the use of NIV or the progression of their disease. Monitoring sleep quality in ALS patient should be part of the multidisciplinary care of ALS/ MND patients. Sleep complaints should be promptly and adequately addressed to improve quality of life of ALS/ MND patients.

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P39 A NEW SCALE FOR MEASURING FATIGUE IN MND: THE LINEAR FATIGUE INDEX

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Background: Fatigue is a common but often overlooked symptom in patients with motor neurone disease (MND); it is associated with reduced quality of life. In order to quantify the problem of fatigue, a high-quality, disease-specific measure is required, however, such a measure, for MND, does not exist.

Objective: To design a self report scale which would measure the symptom of fatigue in MND by generating data that would fit the Rasch Measurement Model. The Rasch model satisfies the axioms of fundamental measurement and is the only way to convert ordinal scale scores into interval level data, suitable for parametric analysis.

Method: Scale items were based on themes and phrases derived from semi-structured interviews on fatigue in six patients with MND and appraised by an expert panel of neurologists, sleep physicians and therapists. A 52 item scale, with a four-point Likert response option, was generated. The questionnaire was administered by mail, or during clinic visits, to MND patients of any age, gender and disability who were under the care of a regional neuroscience centre in the UK.

Results: From the qualitative phase, fatigue was defined as reversible, motor and cognitive impairment with reduced motivation and desire to rest, either appearing spontaneously or brought on by mental or physical activity, humidity, acute infection, and food ingestion. It was relieved by daytime sleep or rest without sleep and occurred at any time but was usually worse in the afternoon. This was the underlying construct of the scale. Pilot data from 55 respondents (54.5% response) were analysed. Mean patient age was 62 yrs, mean disease duration was 4.7 yrs, 71% of respondents had bulbar or respiratory involvement. The Rasch analysis revealed that all items had ordered category thresholds. Items with misfit to the Rasch model, including those displaying differential item functioning for clinically important person factors, were discarded. A final, 11 item scale was produced. Items had a wide location range of 8.5 logits and were well targeted to person scores, there being negligible floor and ceiling effects. Stringent, post hoc tests of unidimensionality were satisfied. External comparison revealed moderate correlation with the Fatigue Severity Scale.

Conclusion: The resultant scale was shown to fit the Rasch model and could therefore be assumed to both measure a unidimensional construct of fatigue and generate interval level data. The self-complete scale had specific validation for MND patients and was both simple and quick to complete. It appears suitable for use in both a clinical setting and as an outcome measure in clinical trials. Further validation on a larger population sample is planned.

P40 MODAFINIL FOR TREATMENT OF FATIGUE IN ALS PATIENTS: PILOT STUDY

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Background: Fatigue is common in ALS patients (1). It can lead to restrictions in work or social events, and otherwise diminish quality of life. The evidence base evaluating treatments for fatigue is small.

Objectives: This pilot study was designed to evaluate the evidence of initial efficacy and safety of modafinil, a medication marketed for the treatment of narcolepsy and sleep apnea, to treat fatigue in ALS patients.

Methods: A 4-week double blind placebo controlled trial of modafinil was followed, for modafinil responders, by an

additional 8 weeks of open label treatment. Placebo non-responders were offered up to 12 weeks of open label modafinil, depending on clinical response at week 4. Active-placebo ratio was 3:1, intended to elicit more objective outcome assessments than provided by open label treatment. Inclusion criteria included ALS diagnosis by modified El Escorial criteria, FVC 50+%, ability to communicate verbally or with an assistive device, English speaking, able to swallow capsules, and clinically significant fatigue, defined as scores of 4.5+ on the Fatigue Severity Scale (FSS). Exclusion criteria included untreated hypothyroidism, untreated hypertension, anemia, or major depressive disorder. The primary outcome measure was the clinician-rated Clinical Global Impressions (CGI) scale based on the self-report Fatigue Severity Scale, Epworth Sleepiness Scale, Beck Depression Inventory, as well as 10-point and visual analog scales. Side effects were assessed weekly, and a brief neuropsychological battery was administered at baseline and week 4. Because of geographic distance and transportation problems, mandatory visits occurred at baseline and monthly, with interim telephone calls.

Results: To date, 15 patients have reached a study endpoint and 5 are currently enrolled in the double blind phase. Three patients randomized to modafinil dropped out because of side effects (tightness in chest (N=1), headaches (N=2). Twelve patients completed the 4-week trial. Based on clinician ratings, 7 of the 8 modafinil patients had a positive response; all 4 placebo patients were non-responders. At baseline, mean FSS scores were 50.6 and 51.6 for future responders and non-responders, respectively; at week 4 their scores were 29.7 and 44.6 respectively (p=.06). There were trend improvements favoring patients randomized to modafinil on Visual Analog Scale ratings of stamina and sleepiness. In addition, there was a trend reduction in self-rated depressive symptoms among patients on modafinil, although mean scores for both groups were in the ''not depressed'' range both at baseline and week 4.

Conclusions: This small pilot study suggests that modafinil may be helpful for fatigue in patients with ALS. However, ALS patients may be more sensitive to side effects than medically healthy patients or those with other medical conditions, so that an initial low dose and slow dose titration are indicated.

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P41 RADIOTHERAPY OF SALIVARY GLANDS FOR DROOLING REDUCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Objective: Assessing the effect on drooling of external radiation of salivary glands and the subjective satisfaction of amyotrophic lateral sclerosis patients.

Background: Drooling and subjective sialorrhea are common in amyotrophic lateral sclerosis with facies-lingual-pharyngeal paresis, in particular in later stages of the disease. The symptoms can cause considerable discomfort and are currently difficult to treat with anticholinergic drugs, which often give rise to side effects. Production of saliva can be reduced with radiation of parotids and submandibular glands. We studied retrospectively patients who underwent external radiotherapy to determine the lowest effective dose of radiation necessary.

Methods: Between August 2001 and Febuary 2006, 14 patients underwent external radiation for drooling. ALSFRS score for salivary was 0 for more than 85% of patients and 1 for the others. Mean age of patients was 62 years old (39–81) and sex ratio was 0.4. All patients were previously treated with anticholinergic drugs with unsatisfactory results. Parotid and submandibulary gland irradiation was performed on each side for all patients except two. Mean surface area of radiation was 80cm^2 (20.25–125.7). Dose of X rays was 5.5mV for 12 patients associated with electrontherapy (9–10MeV) for two. Two patients were treated with electron therapy alone with a dose of 10meV. Response was evaluated subjectively by patient, relatives and caregivers. Total and partial reduction of salivary emission at control schedule was evaluated as a satisfactory response.

Results: Mean duration of follow up was 7 months (0–20). Nine patients died, 2 were lost, and 3 were alive. Satisfactory response was recorded for 57% of patients 3 month after radiation. Total mean dose performed was 20.37Gy (13–32) for responding patients and 14.5 Gy (3–33) for the others. A total dose over 16 Gy seems to be more efficacious (p=0.1).
Four patients (28.6%) suffered local pain which resulted in stopping radiation for two. These two patients were treated with X rays.

Conclusion: External radiation of salivary glands is effective in reducing drooling satisfactorily. A total dose of 20 Gy performed in 5 sessions with electrontherapy may be a good compromise between efficacy and tolerance.

P42 EFFECT OF SHORT-TERM INTERVENTION OF PHYSICAL THERAPY FOR EARLY STAGE AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the upper and lower motor neuron symptoms. Since there is no definitive treatment of ALS, symptomatic and palliative therapy may improve quality of life (QOL). However, physical therapy (PT) has not been well analyzed in the early course of ALS.

Objectives: The aim of the present study is to clarify the effect of a short course of PT intervention in individuals with ALS. In particular, we focused on the activity of daily living (ADL), QOL, falling, gait velocity and depression using several batteries for the early stage of ALS.

Methods: We enrolled six ALS patients who were 'definite' according to the El Escorial criteria. They were ambulatory and not supported by mechanical ventilation. The clinical presentations of the patients were divided into classic (3/6) and pseudo-polyneuritic form (3/6). The ages at onset and disease duration were 64.6 ± 13.5 years old and 17.3 ± 6.2 months, respectively. PT was carried out by physical therapists for 10 days (60 min/day). The program was composed of multidimensional exercises such as neuromuscular re-education of the trunk and lower extremity, dynamic balance (balance ball and sling) and light loads endurance (recumbent ergometer). In order to evaluate the effect of our program, we carried out measurement of the following five scales before and after the course of program; 1) ALS functional rating scale-revised (ALSFRS-R) for ADL, 2) ALS Assessment Questionnaire (ALSAQ-40) for QOL, 3) ten meter walk test for gait, 4) Center for Epidemiological Studies-Depression Scale (CESD) for depression and 5) Falls Efficacy Scale (FES) for confidence in ambulation. Wilcoxon signed-rank test was used for statistical analyses. All data were presented as mean $\pm$ SD.

Results: There was no difference in ALSFRS-R score of the 6 individuals (37.1 ± 5.5) before and after PT. The total score of ALSAQ-40 was improved from 118.8 ± 37.0 to 107.5 ± 32.1 ($p < 0.05$). In particular, the mobility and emotional functioning were significantly improved out of five scales. FES was also improved in six patients (48.8 ± 28.8 to 59.1 ± 30.2, $p < 0.05$). Although the mean scores of gait velocity (34.7 to 42.3m/min) and CESD (20.3 to 10.3) were improved after the PT, there were no statistical differences in both scales.

Discussion and conclusions: The present study revealed that a short course of PT leads to an improvement of mobility, falling, as well as emotional functioning for individuals with ALS in the early stage. PT may also provide some improvement of gait velocity and depression. Although our study was carried out on a limited number of patients, we believe that PT has beneficial effects for the early course of ALS. This research was supported by Health Labour Sciences Research Grant and Sasakawa Hearth Science Foundation.

P43 FALLS IN AMYOTROPHIC LATERAL SCLEROSIS: THE ALS FUNCTIONAL RATING SCALE AND MANUAL MUSCLE TESTING AS PREDICTORS

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Background: Falls, which are sometimes serious, are common adverse events in patients with ALS. The ALS Functional Rating Scale (ALSFRS-R) and manual muscle test (MMT) are standard in ALS clinical evaluation, yet their utility in fall risk assessment is unclear.

Objectives: To assess the value of clinical tests in predicting falls in ALS.

Methods: We used data from 470 patients enrolled in a clinical trial of minocycline. Patients were assessed monthly on several ALS-specific tests for 13 months following enrollment. All falls were recorded as nonserious adverse events. We used a Cox proportional hazard model to test whether baseline (enrollment) values of ALSFRS-R, FVC and MMT (34 individual muscles as well as composite scores of subsets) could predict time to occurrence of first fall for patients. Factors tested included sex, age, symptom duration, bulbar vs. limb onset, weight, body mass index, and riluzole use. We also investigated the predictive ability of individual muscles tested 30 to 60 days prior to the fall in patients matched to controls of the same age and sex who had no falls.

Results: 172 patients (37% of the 470 enrolled) experienced 1 to 12 falls with an average of 2.6 falls per patient for those that had falls during the trial. The median time to first fall was 127 days (range 8 to 404 days) after enrollment. The best predictor of whether a patient would have a fall was baseline ALSFRS-R ($p=0.019$). None of

the other factors, including those evaluated 30 to 60 days prior to the fall, were significant predictors of a subsequent fall. The gross motor subscale consisting of three movements (turn, walk and climb; $p=0.008$) was responsible for the significance of the ALSFRS-R as a predictor. This translates to a 16% difference in the probability that the patient will have a fall during the next 12 months when the median is used as a cutpoint. Half (50%) of those with scores below the median (score $\leqslant 7$) had one or more falls compared to one-third (34%) of those with scores above the median (score >7). None of the other ALSFRS-R subscales were significant predictors. Baseline ALSFRS-R was also a significant predictor of the number of falls in patients who had falls ($n=172$; $p=0.016$). The fine motor movement subscale (handwriting, cutting and dressing; $p=0.015$) predicted number of falls, while other subscales did not.

Conclusions: The gross motor subscale of the ALSFRS-R may prove useful in predicting incidence of falls in patients with ALS, while the fine motor subscale predicts frequency among those who fall. Whether these scales will be as useful as the timed walk or the Timed Up and Go test, deserves further study.

P44 ABOUT FACTORS OF ALS PATIENTS WHO CANNOT WALK: THE INFLUENCE ON FALLING

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Background: The purpose of this study is to research the reasons why all ALS patients have walking difficulties. Though it is suggested that muscular deterionation causes walking difficulties, little is known about factors.

Objectives: From 2001 to 2004 in Kohnodai Hospital, 34 ALS patients who had lost the ability of walking independently were studied.

Methods: We observed the patients from onset of ALS until losing the ability to walk. The following subjects were checked: 1) Time of independent walking for Upper Extremity palsy (UEP) patients, Lower Extremity palsy (LEP) patients and Bulbar palsy (BP) patients. 2) The reasons for walking difficulties. 3) Percentage of wounds from falling. 4) Comparison between falling history and disease types (UEP, LEP and BP).

Results: Independent walking disability is caused not only by the muscular deterionation but also by dyspnea and falling. The characteristics of each disease type are shown: UEP indicates longest possible walking term, LEP had the highest falling risk, in BP dyspnea caused walking disability.
1 case report: ALS patient (male, 78 years old, UEP). He lost his ability to walk through falling and dyspnea. We treated with tracheotomy ventilation and physiotherapy. After 7 weeks of treatment, he recovered his ability to walk independently. Twenty months later, he kept this ability.

Discussion: With this study, we finally recognized the necessity of physiotherapy for ALS patients even after loss of walking ability, because not only loss of muscle power but also dyspnea and falling are important factors. If we can suggest proper treatment, they have a chance to recover walking ability. Physiotherapy is needed not only for delaying this disease but also for improvement of walking ability.

P45 COGNITIVE PROFILES IN CORTICOBULBAR- AND CORTICOSPINAL–ONSET AMYOTROPHIC LATERAL SCLEROSIS: PRELIMINARY FINDINGS

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Background: Although amyotrophic lateral sclerosis (ALS) has been traditionally described as a progressive motor neuron disease resulting in debilitating motor dysfunction and disability, neuropsychological investigations have highlighted the presence of cognitive deficits in a certain subset of patients. There is a consensus that corticobulbar-onset patients are at greater risk for developing global cognitive deficits while corticospinal-onset patients appear to experience more subtle deficits.

Objectives: To evaluate cognitive function, physical status and mood in patients with corticobulbar-onset and corticospinal-onset ALS.

Methods: To date, we evaluated 22 patients with corticobulbar- ($n=7$) and corticospinal- ($n=14$) onset ALS on a comprehensive battery of neuropsychological tests measuring verbal and visual memory, executive function, working memory, visuoperception, language, and attention. Frontal behaviour problems including apathy, disinhibition and executive dysfunction were measured using the Frontal Systems Behavioral Scale (FRSBE). Symptoms of depression were measured using the Beck Depression Inventory-II (BDI-II) and ratings of physical status and motor function were assessed using the ALS Functional Rating Scale (ALS FRS), the FVC score and the dynamometer.

Results: There was no significant difference between the groups on age, education, duration of disease, Full Scale IQ rating and depression. As well, significant differences were not observed on measures of physical status and motor function with the exception of ratings indicating greater disability on the ALS FRS for the corticobulbar group, namely on items measuring speech and swallowing. Cognitive testing revealed that both groups had mild difficulties with learning a list of unconnected words (RAVLT) as well as with perceptual organization (Block

Design). Both groups showed moderate impairments for object-naming (Boston Naming Test), planning (Tower of London), word fluency (Controlled Word Association Test) and complex ocular scanning (the Symbol Digit Modalities Test). Only the corticospinal group showed a mild deficit on the delayed recall and recognition of a word list (RAVLT). Self-ratings and family/caretaker ratings on the Apathy subscale of the FRSBE were abnormally elevated following disease onset as compared to premorbid levels, in both groups.

Discussion: Using a comprehensive and standardized battery of tests, we investigated function in patients with symptoms of either corticobulbar or corticospinal ALS. Preliminary findings revealed that similar and significant cognitive deficits exist in both corticobulbar and corticospinal groups, namely impairments in verbal memory, language (object-naming, word fluency) visuoperceptual organization, planning, and complex ocular scanning.

P46 NEUROPSYCHOLOGICAL DEFICITS IN AMYOTROPHIC LATERAL SCLEROSIS: A SOUTH INDIAN EXPERIENCE

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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 56% of ALS patients experience cognitive deficits which may be identified early in the course of the disease. Cognitive deficits being an integral part of the disease has 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.

Patients & Method: Cognitive function was studied in 18 patients (mean age 31.67 ± 13.62) with confirmed ALS. A neuropsychological test battery comprising tests for attention, executive functions as well as verbal and visual learning memory was administered. In all 21 test 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 included deficiencies in visual attention, working memory, cognitive flexibility, response inhibition, planning, problem solving, and visual-perceptual skills, 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: In conclusion ALS is a disease that affects higher cognitive frontal functions, especially the executive functions.

P47 PREVALENCE AND PATTERNS OF COGNITIVE IMPAIRMENT IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN KOREA

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Background: Traditionally, ALS is considered to be largely restricted to motor neurons. The clinical or pathologic involvement of non-motor systems has been held to be rare or restricted to specific variants of the disease. However, the contemporary view of ALS now encompasses a more widespread disorder in which motor neurons are selectively vulnerable but in which non-motor involvement can also be observed. The occurrence of cognitive impairment in ALS is one such non-motor phenomenon.

Objectives: To investigate the prevalence and patterns of cognitive changes associated with sporadic amyotrophic lateral sclerosis (ALS) using neuropsychological batteries.

Methods: Patients with sporadic ALS (35 male, 18 female) with clinical and electrophysiological evidence of combined upper and lower motor neuron involvement in at least one region (Revised El Escorial Criteria for clinically probable or definite ALS) and healthy age-matched controls (19 men, 12 women) were recruited. Depression was evaluated with the Geriatric Depression Scale, and cognitive function with the Korean version of Mini Mental State. A battery of psychometric tests (Wisconsin Card Sorting Test, the Trail-making test, the Boston Naming Test, Written Verbal Fluency Test, Spoken Verbal Fluency Test, Category Fluency Test, Seoul Verbal Learning Test, Rey-Osterrieth Complex Figure Test and Digit Span) was used to assess memory, visuo-spatial function, attention and executive function.

Results: On non-motor, non-speed-dependent tasks, 48% of patients with ALS had evidence of cognitive impairment compared to 4% of controls. Cluster analysis suggested four patient subgroups: 52% intact, 31% with mild impairment, 13% with moderate impairment, and 4% with severe impairment. ALS patient subgroups, excluding the intact group, performed significantly lower on tests of

executive function than normal controls. Patients with more severe disease also had deficits in confrontation naming and memory. Cognitive impairment was correlated with Norris ALS scale and duration of motor symptoms. Cognitive impairment was not correlated with depression scores. Patients with bulbar-onset ALS were more vulnerable to cognitive impairments than limb-onset ALS and cognitive impairments were more prominent in patients with pseudobulbar palsy.

Conclusions: These data confirm the presence of cognitive impairment in 48% of patients with ALS and particularly implicate executive dysfunction and mild memory decline in the disease process. In later stages of ALS patients had further cognitive involvement. The pattern of cognitive impairment suggests the dysfunction of the frontal network.

P48 WHAT IS THE BEST VERBAL BEDSIDE TEST TO SCREEN FOR COGNITIVE IMPAIRMENT IN ALS?

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Background: The diagnosis of cognitive impairment in ALS (ALSci) typically relies on detailed neuropsychological assessment. This methodology requires several hours and may be impractical where neuropsychologists are not available. Some centers utilize a strategy of screening with a single measure, often letter fluency, and perform detailed testing on patients who fall below a specific cut-off. It is important to understand how practical this screening strategy is and whether any verbal bedside measure can predict overall ALSci.

Objectives: To determine how well specific cognitive measures predict ALSci, and which raw score cut-off has the highest accuracy.

Methods: Neuropsychological assessments were performed on 65 patients at a multidisciplinary ALS center. Patients with major depressive disorder or other neurologic conditions affecting cognition were excluded. Patients completed three to five executive measures and ALSci was considered present in patients with scores of 1.5 or more standard deviations below the mean on two or more measures. Logistic regression determined which measures correlated best with our definition of ALSci. Then, a classification model using ROC analyses was performed on measures correlating with ALSci at or below $p=0.05$ to determine which test predicted impairment with the fewest errors (greatest diagnostic accuracy) based on a specific raw cut off.

Results: Fifty one percent of patients met criteria for ALSci. ALSci did not correlate significantly with demographic or disease-related variables (age, gender, FVC, ALS-FRS score, site of onset). Several neuropsychological tests correlated with overall ALSci by logistic regression, with the following raw scores being most significant: F words ($p=0.003$), S words ($p=0.006$), Digits Backwards ($p=0.0011$), Animals ($p=0.0011$) and A words ($p=0.0013$). Once the predictors were identified, subsequent analysis using ROC classification showed that a raw score of less than 8 F words per minute made the fewest errors in predicting ALSci (accuracy: 79%; specificity: 88%; sensitivity: 73%). The performance was not improved by adding more tests. Digits Backwards had the second highest accuracy (accuracy 74%, specificity 61%, sensitivity 86%).

Discussion and conclusions: To our knowledge, this is the first study to systematically assess the utility of specific bedside neuropsychological measures as a cognitive screen in ALS. Letter fluency (F words) was the best measure for predicting ALSci according to our model, supporting its role as a screening tool with a cutoff of less than 8 words/minute. This simple bedside test improves the prediction over the roughly 50% prior probability at baseline and alerts clinicians to probable cognitive impairment. Digits backwards was moderately accurate but lacked specificity. The effect of moderate-severe bulbar involvement on verbal fluency screening will be discussed.

P49 A COGNITIVE SCREEN OF FRONTAL DYSFUNCTION IN ALS WITH DIFFERENTIATION OF SUBTYPES

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Background: In the evaluation of ALS in the presence of frontal dysfunction (FD), semantic errors for high frequency words during administration of the New Adult Reading Test (NART) have been associated with the frontotemporal dementia (FTD) sub-type of semantic dementia (SD). We recently demonstrated the sensitivity of a 20-minute screening evaluation to detect a relationship in ALS between declines in letter fluency and problem solving (abstract reasoning and judgment). Addition of the NART to this assessment may allow for detection of the surface dyslexia associated with SD, without adding an appreciable amount of time to the clinic evaluation.

Objective: To evaluate the utility of the New Adult Reading Test in identifying declines in semantic ability in ALS patients with frontal dysfunction.

Methods: Twenty minute cognitive screening evaluations were performed on 110 patients in our multidisciplinary ALS clinic. Thirty-five patients of non-bulbar onset and 18 patients of bulbar onset who demonstrated deficiencies in one or more of the Neurobehavioral Cognitive Status Exam (COGNISTAT) subtests of reasoning (similarities and judgment) were administered the NART. NART

findings were compared to results of letter fluency (CFL) and category fluency (animals) and COGNISTAT reasoning measures for both COGNISTAT intact (non-bulbar N=62, bulbar N=26) and COGNISTAT impaired groups, as well as the total sample. Pearson product moment correlational analysis was applied within each group.

Results: Total Sample: NART results correlated with letter fluency for both non-bulbar ($p<.0001$) and bulbar ($p<.008$) onset, and with COGNISTAT similarities ($p<.0001$) for the non-bulbar onset group. COGNISTAT Intact: NART results correlated with letter fluency for both non-bulbar ($p<.001$) and bulbar ($p<.032$) onset groups. COGNISTAT Deficient: NART results correlated with both letter fluency ($p<.001$) and COGNISTAT similarities ($p<.010$) for the non-bulbar onset group.

Conclusions: Numerous screen exams to evaluate for the presence of FD in ALS are now available. Letter fluency continues to be the most reliable marker of FD in screen evaluations, while category fluency is considered to be a marker for progression into semantic dementia. To our knowledge, our screen exam is the first to demonstrate the potential for identifying both the general declines in reasoning that underlie FD, and the specific disruptions to the semantic knowledge base characteristic of the semantic dementia subtype of FTD, with a measure more sensitive than category fluency. Our screen exam remains a preliminary approach to diagnosis, with need for referral for more extensive evaluation in the event of deficient range findings. However, it represents a practical approach to clinic assessment, allowing for greater understanding of the needs of the affected patient, particularly when relatively preserved articulation and fluency of speech may obscure the nature and severity of the patient's language comprehension difficulties in the course of treatment planning.

P50 DEVELOPMENT OF A COGNITIVE BEHAVIOURAL SCREEN FOR USE WITH ALS PATIENTS: PRELIMINARY DATA

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Background: Screening for cognitive and behavioural impairments is important in clinical settings where neuropsychologists are unavailable, when time and resources for detailed testing are limited, or when collecting longitudinal data to demonstrate change is needed. The ALS Cognitive Behavioral Screen (ALS-CBS™) is an assessment tool, developed in our center, containing a compilation of tasks and questions sensitive to frontal lobe dysfunction. The cognitive portion consists of 5 domains specific to frontal lobe function, while the behavioral portion consists of 8 caregiver-directed questions assessing change since disease onset. The ALS-CBS is a 5-minute screen completed in a routine clinical setting by members of the care team.

Objectives: Report preliminary data on the use of the ALS-CBS.

Methods: The ALS-CBS was administered to 150 consecutive ALS patients at three multidisciplinary centers. Patients underwent direct testing by a staff member for the cognitive score, while the behavioural portion was simultaneously completed by the caregiver. Screens were reviewed by the lead neuropsychologist for accuracy and consistency. Analyses included: 1) Comparison of cognitive score means and standard deviation between the patient group and a control population; 2) Comparison of screen scores and diagnostic classification based on neuropsychological assessment in a subset of patients (N=24); 3) Determination of the frequency of specific behavioural abnormalities and whether responses to specific behaviour questions predicted lower cognitive scores.

Results: The mean cognitive score among all ALS patients was 16.3 (2.85) (total possible score: 20) versus 18.3 (1.0) in controls. ALS patients with FTD differed significantly from unimpaired ALS patients ($p<0.01$) and from ALSci ($p=0.04$) but not ALSbi ($p=0.17$). Cluster analysis resulted in 91% correct classification using the cognitive score. Two behavioural questions* predicted lower cognitive scores (mean: 13.4 when either item was endorsed versus 16.7 when change was not endorsed on either item). The most common behavioural changes endorsed by caregivers related to decreased frustration/stress tolerance and decreased interest in previously enjoyed activities.
* *Questions predicting low cognitive scores: 1) Shows poor judgment or problems making good decisions? 2) Gets confused or distracted more easily?*

Discussion and conclusions: Our data provide early evidence that a standardized, brief assessment tool for predicting cognitive and behavioural impairment may be useful and valid in the clinical setting. ALS patients scored below controls on the ALS-CBS and scores correlated with subtypes of frontally mediated impairment. Specific behavioural questions posed to the caregiver predicted decreased cognitive functioning. Continued development and validation of the ALS-CBS is currently underway.

P51 THE SCHEDULE FOR MEANING IN LIFE EVALUATION (SMILE) IN ALS PATIENTS: A PILOT STUDY

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Background: Loss of meaning in life is one of the major determinants for wishes for hastened death in patients with

incurable diseases. The Schedule for Meaning-in-Life Evaluation (SMiLE) is a newly developed instrument for the assessment of individual meaning in life (MiL).
In the SMiLE, the respondents list 3 to 7 areas which provide meaning to their life before rating the current level of importance and satisfaction of each area. Indices of total weighting (IoW, range 20–100), total satisfaction (IoS, range 0–100), and total weighted satisfaction (IoWS, range 0–100) are calculated.

Objectives: The objective of this pilot study was to investigate the feasibility and acceptability of the SMiLE in ALS patients.

Results: Twelve ALS patients of the motor neuron outpatient clinic of the Ludwig-Maximilians-University, Munich, took part in the study. The mean IoW was 88.6 ± 12.2, the mean IoS was 77.8 ± 19.1, and the mean IoWS was 80.6 ± 18.8. The instrument was neither distressing (2.0 ± 2.8) nor time-consuming (2.0 ± 2.9) as assessed by numeric rating scales (NRS, range 0–10). The patients listed family (n=9), partner (n=8), friends (n=5), leisure time (n=4), spirituality (n=1), nature experience (n=1), and home/garden (n=1) as important MiL areas. None of the patients listed health.

Discussion: MiL assessment is of increasing importance in palliative care. Administration of the SMiLE appears to be feasible and well accepted in ALS patients. All patients understood the administration procedure and were able to participate. Health status seems to be overestimated in existing instruments. In-group as well as between-group comparisons to patients with other incurable diseases and the influence of age and gender need to be investigated in a larger sample.

P52 SPANISH ADAPTATION OF THE ALSAQ-40 QUESTIONNAIRE FOR ALS PATIENTS

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Background: All studies about ALS carried out up to now have used clinical measures as results, although some instruments have been recently used for the assessment of global health issues in these patients: muscular strength, muscular function and mortality. However, in spite of the excellent qualities of these instruments, a broad assessment of the subjective health status is needed, with regard to illness impact, functioning and well-being of the patients. There are different specific procedures to assess the subjective health status of ALS patients, nevertheless, due to their psychometric characteristics and the level to which they suit to this population, the ALSAQ-40 (1) and an abbreviated version with five items (ALSAQ-5) are considered to be the most appropiate instruments to assess the general health of these patients. As there are not Spanish versions of these Anglo-Saxon questionnaires the aim of this study is the Spanish adaptation of the ALSAQ-40 questionnaire and the assessment of its statistical properties.

Objective: Cross-cultural Spanish adaptation of the ALSAQ-40 questionnaire to assess subjective health status in Amyotrophic Lateral Sclerosis (ALS) patients.

Methods: The ALSAQ-40 questionnaire was adapted and applied in 53 ALS patients randomly selected from different Spanish regions. Reliability assessment and factorial analysis were done in order to determine the dimensions of the scale. The validation was done by means of comparing the ALSAQ-40 questionnaire with two instruments usually used in the assessment of ALS patients; the ALS Functional Rating Scale (ALFRS) and the McGill's Quality of Life Questionnaire (MQOL), comparing the latter with the emotional dimension of ALSAQ-40 questionnaire.

Results: The Spanish adaptation of ALSAQ-40 has nearly the same statistic characteristics as the original version, although there are some differences. The emotional dimension is more heterogeneous and there is a cluster gathering Feeding and Communication dimensions. ALSAQ-40 and ALSFRS questionnaires showed a high correlation, as did the emotional dimension of ALSAQ-40 with the Psychological Symptoms, Life Expectancy, Meaningful Existence and the whole score of MQOL.

Conclusion: Our results show that the Spanish adaptation of the ALSAQ-40 questionnaire keeps the statistical properties of the original English version.

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P53 A PILOT STUDY TO EVALUATE THE RELIABILITY OF ADMINISTERING THE ALS-SPECIFIC QUALITY OF LIFE INSTRUMENT - REVISED (ALSSQOL-R) IN A SELF-ADMINISTERED FORMAT VS. INTERVIEW BASED FORMAT

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Background: We developed the ALS-Specific Quality of Life Instrument-Revised (ALSSQOL-R) to meet the need for a disease specific tool to assess QOL in patients with

ALS. All studies of this instrument to date have involved administration in multidisciplinary ALS clinics. Unfortunately, distance or physical limitations prevent many patients from attending such clinics traditionally used in our research.

Objectives: 1) To determine whether administering the ALSSQOL-R as a self-report measure in either a web-based or a paper-pencil format provides data comparable to that obtained by the interview administration. 2) To determine the appropriateness of using an internet assessment tool with the ALS population.

Methods: We completed a repeated measures study comparing modes of administration of the ALSSQOL-R (interview compared to self-administration). ALS patients completed the ALSSQOL-R and the ALS Functional Rating Scale–R (ALSFRS-R) in interview with a trained data collector during their ALS clinic appointment. Participants were asked to complete the same ALSSQOL-R measure two weeks later and given the choice of using either a web-based format or a paper-pencil format. Participants provided an evaluation of their experience completing the ALSSQOL-R on their own.

Results: We evaluated 40 ALS patients (53.7% male and 46.3% female; mean age 59 years, s.d.=11.4). Mean symptom duration was 58.8 months; median symptom duration was 35.0 months. Mean ALSFRS-R score was 29.8/48. 58.5% of participants chose to complete the ALSSQOL-R using the internet. Paired samples t-test showed no significant differences in the total score of the ALSSQOL-R comparing interview format to self-administered format (p=.910). Total ALSSQOL-R scores from each mode of administration were strongly positively correlated (r=.71, p=.000). Group sizes were too small to complete statistical analyses; however, there did not appear to be significant differences between the self-administered format total ALSSQOL-R scores. A post-survey evaluation revealed scores of 4.75 out of 5 on a standard Likert scale on both ease in access to the website and ease of completing the survey online. The highest percentage preferred to complete the survey online (44%), with the paper-based (28%) and clinic interview (16%) formats being less favoured.

Discussion and conclusion: Web-based or pencil-paper administration of the ALSSQOL-R produced results comparable to those of the interview format, suggesting that they are reliable means of administration. This study suggests patients prefer completing the ALSSQOL-R via self-administration over interview format. We did not ascertain whether interview format was less desirable due to time taken in clinic, or due to other reasons. In future studies we hope to utilize self- administered versions of the ALSSQOL in order to examine and compare QOL of individuals with ALS who attend multidisciplinary clinics and those who do not. The self-administered version may also be helpful for assessing QOL before and after therapeutic interventions.

P54 TELEPRACTICE: IMPROVING ACCESS TO INTERDISCIPLINARY CARE FOR PEOPLE WITH ALS

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Background: Telepractice is the use of multiple telecommunication technologies to improve the access, efficiency and continuity of care for patients in local and remote locations. ALS is a disease that requires continually changing interventions by multiple health care specialists from diagnosis to palliation. ALS clinics, staffed by a team of clinicians with expertise in ALS, are the preferred locations for meeting all patient needs to optimize quality of life for patients and their families. The increasing disabilities associated with the disease progression often make it difficult or not possible for a patient to travel to these clinics. Our ALS Clinic is located in physical proximity to an assistive technology clinic. Collaboratively, these clinics determine and meet patient needs. The location of these clinics within a member site of the provincial telemedicine network allows for easy access to telepractice service delivery. In December 2002, the clinics initiated use of telepractice to meet the multiple, ever changing needs of patients with ALS in remote locations.

Objectives: 1) To provide interdisciplinary care to patients with ALS in remote locations. 2) To optimize functional independence with assistive technologies through disease progression. 3) To utilize telepractice to optimize delivery of care. 4) To assess the cost effectiveness of telepractice.

Methods: Interdisciplinary care of a single patient was monitored over a nine year period. Telepractice was evaluated for its effectiveness as a service delivery model.

Results: The patient's diverse needs were met by continuous use of telepractice by the interdisciplinary team. Use of telepractice was determined to be cost effective for an ALS patient in a remote location. The estimated cost per year for clinic visits was $52,000 Canadian, compared to telepractice at $1600.

Discussion and conclusions: This study considers the advantages and disadvantages of telepractice for the delivery of interdisciplinary care to patients unable to travel to ALS clinics. The results have demonstrated that telepractice is an effective tool for an interdisciplinary team to provide quality care to ALS patients in remote areas.

P55 A CARE PATHWAY FOR PEOPLE WITH MOTOR NEURONE DISEASE NEEDING NUTRITIONAL SUPPORT

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Background: It is widely accepted that for patients with Motor Neurone Disease (MND) with bulbar symptoms, the early siting of an enteral feeding (EF) tube i.e. PEG (percutaneous endoscopic gastrostomy) or RIG (radiologically inserted gastrostomy) optimises care compared to later placement when a patient may be malnourished, dehydrated, or have respiratory insufficiency.
As part of a project to improve the care of people with MND, a multi-disciplinary team (MDT) of professionals examined the care of people needing nutritional support (NS): particularly at when and how to broach the subject of NS with the patient; decision making over PEG vs RIG vs NGT (nasogastric tube) placement; and streamlining the process.

Objectives: To devise a care pathway to expedite the processes of referral and assessment for the appropriate EF tube. To improve the quality of life for patients with bulbar symptoms.

Methods: A task and finish group of MDT members (dietitians, nurse specialists in MND, doctors in palliative care, respiratory medicine, gastroenterology and radiology) examined current practice, and a literature search about best practice informed the new pathway. It was then refined as patients used it. Training needs were identified to enhance the assessment of respiratory function pre-procedure and facilitation of NG tube placement in the community. Documentation of timing and type of tube placement, use of tube (i.e. initially for hydration or immediately for total nutritional support) and the post-placement morbidity was recorded on a database. Patients and carers were asked about their experiences.

Results: An evidence-based care pathway has been developed. Prior to this pathway, PEGs were the only EF tube offered. The pathway has allowed the appropriate use of RIG and NG tubes. Measurement of sitting and lying forced vital capacity has provided a more objective assessment to inform decision making on the most appropriate EF tube.

Discussion and conclusions: This care pathway has drawn together best practice for patients with MND needing nutritional support. We have discussed NS with patients earlier, so that timely, informed decisions can be made, with verbal feedback from patients and carers that this has empowered them and improved quality of life. A DVD resource is being developed to collate patient/carer comments so future patients can gain further insights. Training for the Home Enteral Nutrition Dietitian on NG tube placement for either short-term hydration purposes prior to PEG/RIG placement or to prevent acute hospital admissions at end of life has further improved overall choice. RIG placements are now appropriately offered and the process by which any EF tube is placed has been expedited. This initiative is already being examined by other specialist groups within our trust as an example of good practice.

P56 DEVELOPMENT OF A REGIONAL-ASSISTANCE NETWORK AND A CLINICAL PATHWAY FOR THE CARE OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic Lateral Sclerosis patients need complex multidisciplinary care. A regional network with multidisciplinary units and a clinical pathway could improve this assistance. Clinical pathways consist of assistance plans for certain diseases with a predictable course; these plans are always established in isolated centres, not in multicentre regions.

Objectives: The aim is to develop a clinical pathway capable of organizing and developing uniform procedures for care in a regional assistance network, which is made up of 5 hospitals, from the beginning until the end of the disease.

Methods: In periodic meetings, neurologists from these hospitals and members of the Regional Health Service revised the therapeutic guidelines already published and other documents used in treating ALS.
A regional network was developed based on creating multidisciplinary units and a clinical pathway adapting information collected on the social-sanitary conditions in this region.

Results: A regional assistance network was created for 5 multidisciplinary units. Each unit includes: case administrators, Neurologists, Neumologists, Physiatrists, Physical therapists, Gastroenterologists, Dieticians, Psychologists, Psychiatrists, Social workers and clinical investigators. A services portfolio for better management of the resources among these hospitals was designed.
A clinical pathway was created consisting of a scientific–technical framework which arranges the care in relation to the diagnosis and the treatment, according to the degree of disease progression and a chronogram.
The framework is accompanied by various patient-information documents on the disease, the tests that are

required and a patient evaluation form. The standards are established to reach and to promote a constant improvement in patient care.
A basic and clinical investigation network was also developed for this disease. Clinical trials would take place to evaluate new treatments.

Conclusions: A clinical pathway for the care of the ALS patients in a regional network homogenizes and organizes the attention and care that the patient should receive from the initial symptoms to the end of the disease. This arrangement and homogenization of the attention improves the quality of patient care, diminishes the variability in work protocol and rationalizes the use of the health care resources available.
The network has also increased the investigation of this disease.

P57 EFFICIENCY OF MULTIDISCIPLINARY UNITS AND THE APPLICATION OF CLINICAL PATHWAYS IN THE CARE OF ALS PATIENTS

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Introduction: Amyotrophic Lateral Sclerosis (ALS) requires complex patient-care. Clinical and Social Multidisciplinary Units and Clinical Pathways could organize and give better assistance to ALS patients.

Objectives: The objective is to evaluate the efficiency of the implementation of a multidisciplinary unit following a Clinical Pathway of social-sanitary attention.

Methods: Observational study of patients attending the ALS Multidisciplinary Unit during 2006. Patients were analyzed as they were being diagnosed in the ALS Unit (UDG) or in other departments of Neurology (ODG). Early diagnosis, functional state (ALSFRS-r scale) at the time of the diagnosis, and the treatment, including not only medical but also the psychological and social treatment were recorded. Statistical analysis (T-student and Chi-square) was performed.

Results: Forty-five patients were studied (UDG 10, W4/M6; ODG 35, W19/M16). The average age of both groups was similar (UDG 67.3 yo/ODG 59.67 yo; $p=0.053$). The UDG patients were diagnosed during the early-onset of the disease with less time since the beginning of their symptoms (UDG 9.9 months/ODG 27.61 months; $p=0.002$). A better functional situation was found (UDG 39.44 points/ODG 27.74 points; $p=0.0006$) at the time the disease was diagnosed. There were no differences in the number of gastrostomies done (UDG 10%/ODG 8.6%; $p=0.91$) but there was a difference in the number receiving non-invasive ventilation (UDG 40%/ODG 11.5%; $p=0.017$) in the first two years after the diagnosis. Therefore respiratory support is earlier in UDG patients. Physiotherapy care (88.2% vs. 29.1), psychological (94.1% vs. 16.6%) and social attention (94.1 vs. 33.3) ($p<0.0001$) was greater in UDG patients.

Conclusions: The ALS care given in multidisciplinary units following the Clinical Pathway achieves an earlier diagnosis and earlier patient-care in patients with a better functional state.
The increase and earlier application of medical treatments, respiratory support, physiotherapy, psychological and social assistance allows and achieves an improvement in healthcare quality.

P58 CLINIC AND COMMUNITY: AN INTEGRATED CARE PATHWAY FOR ALS

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Background: Community service providers with a broad service mandate must ensure their staff have adequate knowledge in order to care for their patients. In the case of ALS, this is often a challenge due to the relatively small number of individuals with this disease. An integrated care pathway (ICP) is a multi-disciplinary outline of anticipated care. The Champlain Region ALS Integrated Care Pathway was developed in response to feedback from community service providers who had few patients with ALS and were therefore unfamiliar with the disease. They needed an easy-to-use guide to ALS and its management.

Objectives: The ALS ICP was designed to be a user-friendly guide to educate professional care providers in the community and to enhance their understanding of the disease process from onset to end-of-life issues. The goal was also to promote and support the development of multi-disciplinary care partnerships.

Methods: The Ottawa Hospital Rehabilitation Centre ALS Clinic multi-disciplinary team and the Champlain Community Care Access Centre developed the structure and the content of the ICP. Input was received from community groups including the ALS Society, Hospice at Maycourt, VHA Health and Home Support Attendant Care Outreach Program and families living with ALS.

Results: The ICP is divided into seven areas: mobility, respiratory, communication, cognition, eating and swallowing, nutrition and spirituality. Each area includes background information and describes the stages of progression with presentation, psychosocial considerations, potential risk, interventions and supportive resources. In addition, there is detailed information on key service resources for ALS management including clinic and community programmes, books, and videos.

Discussion and conclusions: The ICP is the result of extensive consultation and collaboration with groups in the community having ALS knowledge and expertise. The ICP is designed to enable professional service providers and non-professional caregivers to recognize problems early, and suggests interventions to manage the problems, thus averting crises. The ICP identifies local community resources that can assist in the management of the many and varied disease-related issues. Used as a planning guide for service providers, the ICP's pro-active approach is expected to benefit patients and their families in securing appropriate resources and supports in a timely manner.
The ICP is intended not to replace professional clinical assessment and support but to complement them. Its purpose is to ensure a consistent continuum of care throughout the course of the disease whether the care is provided by the clinic, community services in the patient's home or at a care facility. Anticipated benefits are increased efficiency and effectiveness of care delivery, decreased need for crisis management and ultimately improved quality of life for our patients and their families.

P59 THE ROLE OF A CLINICAL PHARMACIST IN A MULTIDISCIPLINARY ALS/MND CLINIC

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Background: ALS patients have complicated prescription and non-prescription regimens and can benefit from consultation with a clinical pharmacist who assesses drug-related problems and answers drug-related questions. This project was designed to quantify and describe activities performed by a clinical pharmacist in a multidisciplinary ALS clinic.

Objectives: Identify number and types of medications used by ALS patients; list number and types of interventions performed and types of educational topics discussed by the clinical pharmacist; determine amount of time spent in direct contact with patients and researching drug information questions.

Methods: This prospective, cross sectional, data collection study included all patients seen by a clinical pharmacist as part of the standard care received at a single, quarterly appointment. The pharmacist recorded demographic information, lists of prescription and over-the-counter medications, number and types of pharmacy interventions performed, types of education provided, time spent with the patient, and time spent researching drug information questions at each clinic visit.

Results: Thirty-seven patients were included in the study. The average number of prescription medications used per patient was 3.59 (0–10) with 1.75 (0–9) used for ALS-related indications. Riluzole was used by 40.5% (15/37), and the pharmacist recommended initiating it in 4 additional patients. Over-the-counter medications were used by nearly 90% (33/37) of patients with an average of 3.5 products used per patient. Of these, 1.4 were being used for ALS-related reasons. The pharmacist spent an average of 21 (5–50) minutes with each patient and an additional 3 (0–20) minutes researching questions or calling community pharmacies to obtain accurate drug lists. The average number of pharmacist interventions was 2 per patient, with the majority of recommendations relating to ALS symptoms or monitoring liver function tests for riluzole. The pharmacist provided education on an average of 2.5 topics per patient with the majority of topics ALS-related. However, medication issues not related to ALS were discussed with 43% (16/37) of patients. These topics included: reviewing blood pressure goals, assessing compliance with medications used for cardiovascular disease or asthma, and discussing monitoring requirements and side effects of medications used for non-ALS related indications such as warfarin.

Discussion and conclusions: A clinical pharmacist contributes to the multidisciplinary team in the following areas: 1) allows more time for the neurologist to attend to neurologic issues; 2) counsels patients about side effects of ALS-related drugs (prescription and over-the-counter; 3) investigates over-the-counter products for side effects and drug interactions; 4) discusses general medicine issues and likely spends more time with patients than primary care providers; 5) orders prescriptions with minimal errors. Our clinical pharmacist is supported by the hospital and is a member of the neurology clinic staff.

P60 DURATION OF CAREER AS A COORDINATOR AND THOUGHTS OF ATTENDANCE AT A BAD-NEWS TELLING SESSION FOR ALS PATIENTS IN JAPAN

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Background: A study group for improving community medicine for patients with intractable diseases (supported by the Ministry of Health, Labor and Welfare) planned to make a manual for coordinators to be consulted for patients with intractable diseases (mostly neurodegenerative disease and especially focusing on ALS) in 2005. As members of the study group, we needed to know the actual

situation of coordinators working for patients with intractable diseases in Japan.

Objective: We aimed to ascertain the occupational background, duration of former career and coordinator experience, and thoughts on attendance at a bad-news telling session from a doctor to patient/family member(s).

Methods: We sent an e-mail with a questionnaire to the coordinators who were working under each prefectural government in Japan, in November 2006 and retrieved the reply by December 2006. At that time there were 35 coordinators in 28 prefectures designated by each prefectural government. Two of the authors (YN, MN) judged independently their view points divided into two groups, positive and not-positive, about attendance of a coordinator at a bad-news telling session, after reading the answers to the open question. In case of discordance of judgement, authors would discuss and settle the view point into either group.

Results: We retrieved the responses from 24 coordinators (retrieval rate: 68.6%). Fifteen of 24 responders were nurses, 7 were public health nurses and 2 were medical social workers. The duration working as a coordinator was from 0.1 to 8, 3.2 +/− 2.1 (mean +/− SD) years. They had each previous careers from 4 to 35, 16.8 +/− 9.0 (mean +/− SD) years. There were 17 coordinators who had experiences of attendance at a bad-news telling session. Seven had no experience of such a session. The positive group had a shorter duration as a coordinator (n=11, 2.4 +/− 0.56 years) comparing with the not-positive group (n=12, 4.1 +/− 0.53 years) with p=0.036 by t-test.

Discussion and conclusion: Though the coordinators had large diversity as to their positions and income depending on each local situation, most of their occupational backgrounds were nurses or public health nurses working as part-time staff, and who had relatively good experience of former careers. This survey showed a relatively short duration as a coordinator was associated with a positive view point on attending a bad-news telling session. The retrieval rate of this study was not so good (68.6%), but it may be inferred that they became a coordinator with a lot of positive interests and activity, then they become wasted gradually by a shortage of sufficient supporting staff and care by other disciplines, or that the new coordinators felt it necessary to attend such a session due to a shortage of other supporting healthcare professionals.

P61 EDUCATIONAL NEEDS TO OVERCOME DIFFICULTIES THAT NURSES EXPERIENCE IN CARING FOR INDIVIDUALS WITH ALS/MND

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Background: Patients with progressive neurological diseases experience diverse and complex difficulties and suffering. The nurses who share the suffering of these patients and offer them palliative care also undergo such emotional difficulties as hardship and anguish.

Objective: To describe the hardship and anguish experienced by nurses and extract educational elements that may be used to improve individual nursing capabilities when dealing with psychological support.

Methods: Survey 1: The postal survey was administered to those nurses who were employed at one of 16 hospitals. The questionnaire covered major difficulties and conflicts that they had experienced. Survey 2: A survey similar to 1 was conducted on 11 public health nurses taking charge of intractable diseases. Survey 3: The records of the consultation that the ALS/MND nurse coordinator had with nurses were analyzed.

Results: Survey 1: Responses were returned from 27 nurses (Response rate 56%). The following were cited as the most unforgettable scenes: 1) in nursing a patient who is dismayed when forced to make a decision; 2) in facing a patient who is grieving over the unrelenting progression of his illness; 3) in confronting the suffering of a patient who was incapable of communicating; 4) in evaluating the palliative care while preparing for the final moment; and etc. Survey 2: Ten responses were obtained (Response rate 91%). The most unforgettable scenes relative to suffering of family members were: 1) the question of whether family members should tell the patient the true truth of his illness; 2) lamentation on exacerbation of the illness; 3) regret after the patient's death; and etc. The scenes by which they were saddened most in relation to the patient's mental suffering included: 1) when the patient wished to die; and 2) when the patient confessed that he was not ready to accept the diagnosis. Survey 3: Most of the consultation was with public health nurses and home visiting nurses, but few with hospital nurses. The content of consultations included: decision-making made on therapeutic modalities; problems with family relationship; and so on.

Discussion: The following educational elements were considered necessary for individual development of nurses: active listening, importance in maintaining a psychological distance from the patient, method for coaching patients, understanding patients, care of families, ideal approach to support the patient in decision making, debriefing, and stress management for the nurses. It is necessary to validate these elements in future.

P62 THE CREATION OF AN INTERACTIVE BRAZILIAN ORIENTATION SOFTWARE FOR AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Objective: The aim of the study was to elaborate a new strategy from a computer program (Brazilian Software) to facilitate orientation in the rehabilitation process of amyotrophic lateral sclerosis (ALS) patients.

Method: Elaboration of the product Mibrela (Brazilian Interactive Manual for ALS), a Brazilian software for orientation of ALS patients that was based on a printed manual previously elaborated by the authors from this study and applied to 114 patients with a probable or definite ALS diagnosis cared for in the Occupational Therapy Service of the Neuromuscular Disease Sector – UNIFESP/EPM.

Results: This manual consists of a CD (*compact disc*) that is applicable to the screen desktop. It requires just one simple computer without Internet access, but with some programs inside: .NET *Framework* 1.1 and a *Windows* operating system (98/ME/2000/XP). The program has 102 options of statements, including pictures, definitions, explanations of some products and equipment (i.e. splints, bath chairs), energy saving orientations, seating and ergonomic changes at home to increase the ALS patient's independence and security.

Conclusion: This software presents didactic information to facilitate orientation for ALS patients, helping them to increase independence in daily living activities and improving quality of life.

P63 WHO JOINS PATIENT ORGANISATIONS AND WHY? A QUESTIONNAIRE STUDY IN PEOPLE WITH MND

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Background: There has been an increase in popularity of patient organisations in recent years (1). As well as providing their members with a range of social and emotional support, patient organisations are a means for members to highlight deficiencies in services and hence are an important representative of the patient's "voice" (2). However, limited empirical research has been carried out to determine the benefits of membership or the reasons why some patients become members and why others prefer not to join.

Objectives: To ascertain from people with MND whether they joined their representative patient organisation, or have been members in the past. To determine the reasons why people become members or conversely fail to join and determine patient characteristics and patterns of membership.

Method: A questionnaire survey of 87 people with MND, randomly selected from a hospital database. Consenting patients self completed a brief anonymised postal questionnaire. Results presented below are those people responding within the first 4 weeks.

Results: Forty-two patients responded (48%), and 33 sent back completed questionnaires (38%). The majority of respondents are male (76%), married (88%), White British (94%), retired (61%) and considered themselves disabled (82%). Just over half of the population (55%) are current members of a patient organisation, 9% had been in the past and 36% had never taken up membership. 83% of members are male and 50% were over 65. Eighty three percent of members considered themselves disabled. Over half those who had joined were also members of other groups and clubs (56%). Only 25% of patients newly diagnosed in 2007 had joined a patient organisation. The main reason for choosing to join was the "provision of a source of support" (25%). Among those who had joined the main benefit of membership was accessing "up to date information on new treatment and research" (33%). The main disadvantage of joining was felt to be "meeting people with a more advanced condition" (53%). The main reason for never joining a patient organisation was "prefer not to be reminded about illness" (17%).

Discussion and conclusions: In contrast to cancer where the majority of patients do not join a patient organisation (3), in our MND population the majority of people do join, chiefly to access support. Their main perceived benefit is learning about research, including new treatments. However, a significant minority either do not join or leave, so the membership may well not be representative of the total patient population.

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THEME 3 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P64 CLINICAL CHARACTERIZATION OF MOTOR NEURON DISEASE: A RETROSPECTIVE STUDY

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Background: The motor neuron disease (MND) constitutes a group of neurodegenerative disorders characterized by loss of motor neurons. They include progressive muscle atrophy, which compromises lower motor neurons; progressive bulbar palsy, which involves injury of upper motor neurons and/or lower motor neurons, primary lateral sclerosis, injuring exclusively upper motor neurons; and amyotrophic lateral sclerosis, resulting in degeneration of upper and lower motor neurons. MND is characterized clinically by spasticity, weakness and hyperreflexia, when upper motor neurons are involved, and fasciculation, atrophy, weakness and hyporeflexia when lower motor neurons are affected. MND's evolution can compromise upper and lower limbs, as well as areas inervated by bulbar nerve fibers. The survival of MND patients is low, and is dependent on the initial involvement.

Objectives: To characterize the clinical profile of the patients with MND treated at the *MND Research Center* at UNIFESP; to determine the relationship between initial manifestations of MND and its definite differential diagnosis; to determine how survival is associated with the various MND profiles.

Methods: This retrospective study included 158 medical records of patients with definite MND diagnosis according to 1994 El Escorial criteria, randomly chosen from the 860 available ones filed in the period from 1990 to 2006. Data collection included age, sex, race, date and site of first MND symptoms, date of registration with *MND Research Center* and date of death. Qui-square of Pearson was used to evaluate differences between the joined ratios. Survival analysis was carried out using Kaplan-Meyer method and Log-Rank test. Statistical significance was set at $p < 0.05$.

Results: The most frequent site of first MND symptoms was the lower limbs (57 patients - 36.1%) and weakness was the most frequently reported symptom. Median survival was 60 months with median time of disease follow-up of 36 months. Median age at dealth was 71 years old. Patients reporting widespread MND symptoms had highest survival rates whereas patients reporting initial symptoms as bulbar manifestation had the lowest survival rates.

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P65 CLINICAL ASPECTS AND PROGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS IN SOUTH WEST CHINA

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Background: Amyotrophic lateral sclerosis (ALS) is the most common and fatal degenerative motor neuron disease in adults.

Objective: To investigate the clinical features, progression and prognosis of patients with ALS in South West China.

Patients and methods: The medical records of all patients diagnosed with adult-onset ALS (El Escorial diagnosis criteria) at the Department of Neurology of West China Hospital of Sichuan University of China (the largest tertiary referral centre providing medical cover in South West China for neurological diseases), between May 2003 and April 2007 were reviewed. Clinical features and the outcome assessed by follow up were collected.

Results: In the study, 88 patients with sufficient information were analyzed. Of them 56 were male (63.6%) and 32 (36.4%) were female with a mean age of 53.4 ± 10.3 years. The mean age at onset was 51.6 ± 11.0 years; the mean delay between onset and diagnosis was 17.8 ± 17.1 months. Four cases (4.5%) had a positive family history of ALS. Only six cases (6.82%) had been exposed to pesticide and insecticide. There were 38 cases (43.2%) with symptoms at onset in lower limbs, 38 cases (43.2%) in upper limbs, 12 cases (13.6%) in bulbar region. Other onset symptoms included paraesthesia in 9 cases (10.2%), fasciculation in 8 cases (9.1%) and cramp in 2 cases (2.3%). The mean survival of the dead group (23 cases, 26.13%) was 21.2 ± 12.2 months. There was no significant difference in gender and initial site of onset between survival patients (upper limbs in 24, lower limbs in 33, and bulbar region in 8) and dead patients (upper limbs in 14, lower limbs in 5, bulbar region in 4) ($P > 0.05$). However, survival patients had an earlier age of onset than dead patients (50.07 ± 11.38 years Vs 56.08 ± 8.03 years, $p < 0.05$).

Conclusions: Our results for mean age of onset, progressive limb weakness being the most common initial symptom, mean survival, mean delay between onset and diagnosis, and longer survival correlating with earlier age of onset was similar to those of other reports. Paraesthesia (10%) as the onset symptom reported here should alert physicians not to exclude the diagnosis of ALS at the very early stage. Further prospective study is needed to

understand better the clinical aspects and outcomes of Chinese patients with ALS.

P66 CLINICAL CHARACTERIZATION AND INTERVENTION IN ALS: A COMPARTIVE STUDY OF TWO COHORTS

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Background: The incidence of ALS is approximately 2.1/100,000. The disease carries a lifetime risk of 1:400**.** Detailed characterization of patient demographics, investigations and interventions is essential to the successful operation of an ALS register, and helps to inform the development of responsive health services. The Register can also be used to retrospectively compare the effects of specific clinical interventions on survival.

Objective: To evaluate the in the quality of information provided to the Irish ALS Register over a 10-year period.

Methods: Using the Irish ALS register, relevant information from the two years of interest, 1995 and 2005 was compared and contrasted. The method sought to identify: differences in the uses and outcomes of interventional procedures; differences in the quality of information available to the Register.

Results: The register identified 55 patients with ALS in 1995, and 70 patients with ALS in 2005. In 1995 there were 31 patients with limb onset disease, 18 bulbar onset, and 4 with generalised onset disease. The clinical onset was not recorded in 2 cases. In 2005, 48 patients had limb onset disease, 2 had bulbar onset, 3 had generalised onset, and in 7 the onset was not recorded. A therapeutic intervention other than Riluzole was undertaken in only 18.8% of the 1995 cohort compared with 30% of the 2005 cohort. Accurate details of the date of disease onset were recorded in only 20% of the patients in the 1995 cohort, compared with 91.4% of the 2005 cohort. The average time taken from diagnosis to intervention in 1995 was 32.4 $\pm$35.3 months, and in 2005 this fell dramatically to 4.29$\pm$ 4.11 months including 2 cases where an intervention was undertaken prior to the establishment of a definitive diagnosis.
In 1995 only 11% of patients underwent gastrostomy, compared with 58% of the 2005 cohort. Non-invasive ventilation was not utilized in the 1995 cohort, while almost 25% of the 2005 cohort has received NIV.
Comparison of survival between the 2 cohorts is currently underway.

Conclusion: There have been significant improvements in the quality and accuracy of information available to the Irish ALS register. The information available from the Irish Register permits detailed analysis of the impact of improved clinical management on outcome, and will assist in health planning for the future.

P67 EFFECTS OF BASELINE LABORATORY VALUES AND MEDICATION USE ON DISEASE PROGRESSION IN ALS

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Background: The range of values for routine laboratory tests in people with ALS are not published. It has been reported that serum chloride and bicarbonate levels are prognostic factors for survival in ALS. People with ALS are on several medications for symptom management and other medical illnesses. It is not known whether commonly used medications affect measures of disease progression.

Objective: The study objective is to examine laboratory parameters and medication usage and their influence on disease progression in a large well-characterized cohort of people with ALS.

Methods: A combined database of 596 volunteers with ALS was generated from three clinical trials and one prospective observational study. Disease progression was measured with the amyotrophic lateral sclerosis functional rating scale (ALSFRS) and vital capacity (%VC). Statistical analysis was performed using SAS (SAS, Cary, NC). Univariate and multivariate analyses for survival were conducted using the Cox Proportional Hazards model to test the prognostic significance of laboratory parameters and medications. A random effects model was used to determine the association of each baseline laboratory measurement or drug with disease progression.

Results: Lower chloride level (HR=0.77, 95% CI 0.6–0.9, p=0.01,) and higher bicarbonate level (HR=1.28, 95% CI 1.0–1.6, p=0.03,) were associated with decreased survival after adjusting for covariates including age, weight, time from symptom onset to screening, and baseline ALSFRS and %VC. Higher bicarbonate (p=0.002) and lower chloride (p<.0001) levels were associated with a more rapid rate of decline in %VC. Lower chloride level was associated with a faster rate of decline of the ALSFRS (p=0.001). Among the various laboratory values, Serum ALT was found to be abnormally elevated in 10.8% of the participants (normal 0–75 u/l) at any time point during the trial.
Cumulative frequencies of common medications used by participants were 58% for vitamin E, 56% for riluzole, 47% for vitamin C, 41% for NSAID and 20% for Coenzyme Q10. Use of statins, riluzole and vitamins did not effect survival or disease progression. Use of Calcium at baseline had improved survival (HR=0.404, p=0.0149) for survival outcome Death, compared with non-users.

Conclusions: Baseline levels of serum chloride and bicarbonate were found to be prognostic indicators of

survival. Use of most medications did not impact survival or disease progression. Since these results are from combined databases of clinical trials for other therapies, it is important to validate the results in other large cohorts.

P68 SURVIVAL TIME IN ALS RELATED TO SITE OF DISEASE ONSET, MOTOR FUNCTION PARAMETERS AND ALSFRS: SIGNIFICANCE OF BASELINE PATTERNS AND THE RATE OF CHANGE DURING A 3-YEAR-FOLLOW UP

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Background: Some epidemiological and clinical parameters at disease onset in amyotrophic lateral sclerosis (ALS) have an impact on prognosis. We found that the decline of vital capacity (VC), ALS functional rating scale (ALSFRS) and muscle strength (MRCCS) over one year are best for predicting survival (1). This was confirmed by other ALS-centers.

Objective: 1) To now clarify whether the rates of decline of VC, muscle strength and ALSFRS predict survival over 3 years; 2) To determine whether a set of criteria including body mass index (BMI), site of clinical onset, baseline VC, MRCCS, ALSFRS, early upper motor neuron (UMN) involvement, MRI pathology found at the first visit can serve as prognostic markers.

Methods: 534 patients with definite ALS followed systematically were included into this retrospective analysis. Baseline values and progression of VC, MRCCS and ALSFRS, and other criteria observed within the first, second and third year after the baseline visit at our center were correlated with survival time.

Results: The average survival time of patients with bulbar onset was significantly shorter (32.8+/−6.8 months) than in the limb onset group (42.2+/−4.8, $p < 0{,}001$). In contrast, the exact site of initial disease manifestation in the limbs was not predictive for survival time. VC, MRCCS and ALSFRS at baseline also failed to have prognostic relevance. A positive correlation to survival was only shown for baseline higher VC in a small subgroup of patients (primary distal upper limb manifestation, $k=0.648$, $p<0.01$). Baseline BMI indicated longer survival ($k=0.182$, $p<0.01$). An early clinical or electrophysiological UMN involvement or evident MRI pathology at the first visit was not predictive of survival time.
Decline of VC within the first ($k=-0.716$, $p<0.001$) and second ($k=-0.55$, $p<0.001$) but not the third follow-up year correlated inversely with survival time. The decrease of MRCCS in the first ($k=-0.53$ to -0.66, $p<0.01$) and second year ($k=-0.49$, $p<0.01$) and the decline of ALSFRS in the first 12 months ($k=-0.48$ to -0.68, $p<0.01$) predicted shorter survival.

Conclusion: While an initial bulbar symptom pattern indicated shorter survival times, no specific limb manifestation correlated with prognosis. Pronounced deterioration of VC and MRCCS in the first and second year and a rapid decline of ALSFRS in the first year had an impact on a shortened residual life-span. Early UMN involvement and MRI findings at baseline examination did not correlate with survival time.

Reference:
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P69 SURVIVAL OF 106 CASES OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

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Objective: To identify the factors related to ALS outcome in a retrospective study.

Methods: We included 106 patents with ALS enrolled in our hospital from January 1994 to December 2004. The patients were monitored with a standard evaluation form, which recorded clinical features, ALSFRS-R and FVC every 3 or 6 months from visit to death or tracheostomy.

Results: Mean age at onset was 52.08 (SD 10.48) years. According to revised El Escorial diagnostic criteria (EEDC), 52 patients had definite ALS, 37 probable ALS, 17 possible ALS. The median survival time from symptom onset was 35 months (95% CI=30 to 40). In univariate analysis, outcome was significantly related to onset site, time from onset to diagnosis and predicted FVC at diagnosis. In the Cox multivariate model, time from onset to diagnosis and predicted FVC at diagnosis were significantly related to outcome. Age, sex, EEDC classification, ALSFRS-R at diagnosis were negative predictors of survival.

Conclusions: Time from onset to diagnosis was a strong predictor of survival, suggesting the progression rate at diagnosis needs to be discussed in the future.

P70 LONG SURVIVAL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: REPORT ON A STUDY OF 1153 PATIENTS SEEN FROM 1958 TO 2005 IN INDIA

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Background: The median survival of ALS patients is reported to be between 23 and 36 months. No report on survival is available from India.

Objective: To evaluate the survival pattern among 1153 patients evaluated at a single Neurological Institute and seen between 1958 to 2005.

Methods: Retrospective analysis of medical records. Some patients contacted by letter and telephone for follow-up. Statistical tools: 2-way cross tabulation, Chi-square test, Kaplan-Meier survival analysis.

Results: A total of 1153 patients were evaluated. 124 patients had expired during follow-up. Men-855; Women-298. Bulbar onset-313 (27.1%); spinal onset-840 (72.8%). El Escorial grouping was: Definite-642(55.7%), probable-442(38.3%), possible-69(6.0%). Age at onset(AAO) categorized as <30years, 30–40, 40–50, 50–60 and >60. Among the dead 96 of 855 were men and 28 of 298 were females. The mean survival duration (MSD) for males was 120.1±6.2months and for women 122.2 ±11.0months. There was no gender difference (p=0.598). The overall MSD was 120.8±5.5months. According to the AAO category, the number of deaths in <30years=3/177, 30–40= 25/211, 40–50=42/306, 50–60=29/291, >60=25/168. The mean survival was 184.5±5.1 months, 108.4±11.6, 86.4±7.0, 98.2± 12.5, 82.5±18.0 respectively. In patients with AAO <30 years survival was significantly higher (p=<0.0001) as compared to all other groups. Similarly AAO >60 years was significantly lower (p=0.025). For deaths among the bulbar onset group 30/313 and limb onset group 85/840, the mean survival was 69.0±7.5 and 131.9±6.0 months (p=0.001). Based on El Escorial categories: Definite-75/642, Probable-42/442, Possible-7/69, the mean survival was 101.3±8.0, 134.8±7.8, 146.8±14.4 months. There was significant difference between definite and probable but no difference between possible and other groups. Survival based on the duration of illness at presentation was ascertained. Mean duration of illness for men was 130.9±7.0 and for women 115.9±10.1 months. No significant gender difference. Duration of illness for AAO categories was 175.7±2.5months, 119.5±15.8, 68.0± 4.3, 65.7±3.7, 49.9± 4.6. AAO <30years had significantly (p-0.001) longer illness duration compared to all the other groups (p=0.025), except 40–50 age category(p=0.10). Mean duration of illness for bulbar onset was 64.1±6.1 months and for limb onset was 136.3±6.4 (p=0.002). For the El Escorial groups duration was: Definite-100.7±6.6 months, Probable-136.5±9.1, Possible-102.8±6.3. The Probable group showed significant difference with the possible group (p=0.001) and trended with the definite group (0.096).

Conclusions: Large numbers of patients with young onset ALS and onset before 30 years had very long survival (15.3 years). Even when age of onset was more than 60 years the survival was indeed long (6.9 years). Overall survival was 10.1 years. Limb onset had longer survival than the bulbar onset group (11.0 vs 5.8 years).

P71 PREDICTORS OF SURVIVAL AND COMORBIDITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive, degenerative disorder of upper and lower motor neurons with a progressive bulbar or limb muscular atrophy. The average survival in ALS patients lies between 3 and 5 years, but the survival in an individual ALS patient is known to have a wide variety and is considered to be difficult to predict. Little is known about the influence of concomitant diseases on survival in ALS patients.

Methods: We identified 479 patients in our ALS Database who fulfilled the diagnostic criteria for probable or definite ALS according to the El Escorial criteria of the World Federation of Neurology and who have regularly been followed at our ALS-clinic in a time period from 1996 to 2006. Demographic, clinical and electro-physiological data were collected at each clinical visit. The ALSFRS-R (ALS Functional Rating Scale Revised) score was performed at each follow-up visit at approximately 3-month intervals for evaluations of disease progression. Major interventions such as non-invasive ventilation (NIV) or percutaneous endoscopic gastrostomy (PEG) were recorded during follow-up. The ''ratio of ALSFRS-R score'' was defined as decrease of the ALSFRS-R score divided by the time interval between two visits. The descriptive analyses and correlation (Pearson test two sided) were performed with SPSS-Software.

Results and Discussion: The study cohort consisted of 479 patients, 269 men and 210 women. The mean age at the time of disease onset was 58 years (SD 12), ranging from 25 to 89 years. 165 of the analyzed patients had died at the time of analysis. Survival from symptom-onset ranged from 4 months up to 11.9 years. Five percent of patients died within 1 year after symptom-onset (age: 43–77 years), 81% (age: 34–85 years) of the patients lived one to five years after first symptoms, 12% (Age: 38–75 years) survived five to 10 years, and 2% lived longer than 10 years after the first symptom.

Older age of onset, bulbar onset, lower initial FVC, shorter time between symptom onset and first visit, lower ALSFRS-R score at first contact, higher ratio of ALSFRS-R score between first symptom and first contact or during the whole disease course correlated with a higher risk of death. In addition we studied the incidence of concomitant diseases in our ALS-patients in comparison with our non-ALS-population. We found that the ratio of ALSFRS-R score between two visits (within 90 to 100 days), no matter at which stage of disease, can predict progression of ALS. This makes this factor easily measured and helpful. Predictors of survival are important for designing new trials and to randomize patients correctly.

P72 PROPORTIONS OF SUDDEN DEATH IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Sudden death in ALS has been described in several studies, especially in end-stage ALS and respirator dependent patients. However the percentage of patients with ALS that die suddenly and unexpectedly is unknown.

Objectives: In this study we investigated the extent of sudden death in ALS during all stages of the disease, and studied possible predictors for dying suddenly and unexpectedly.

Methods: We screened all 302 Dutch ALS patients who once visited our outpatient clinic and who died between 2000–2005. Questionnaires were sent to patients' general practitioners; the response rate was 74%.

Results: Twenty-one percent of the ALS patients had died suddenly and unexpectedly according to the attending general physician that was involved in the last stage of the disease. No significant differences in survival time, sex, site of onset, age at onset and death, smoking habits, alcohol consumption and medical history were found. Variables describing disease progression also did not show a significant difference. Nevertheless, the percentage of patients that were bedridden or wheelchair dependent was lower in the sudden death group, 23% vs. 40%, although this did not reach statistical significance ($p=0.08$). The same applied to arm function, which was better in the sudden death group ($p=0.18$).

Discussion: Approximately one in five ALS patients died suddenly and unexpectedly. This study found no significant difference in patient characteristics between the two groups. Variables concerning disease progression showed that these patients had better motor function just before death than others. The findings of this study suggest that sudden death is not limited to end-stage ALS.

P73 IMPAIRMENT OF AUTONOMIC CARDIAC CONTROL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: ALS is a fatal degenerative nervous system disease caused by death of motor neurons in the motor cortex, brainstem and the spinal cord. It is now recognized that the pathological process is not restricted to the motor system. There is increasing evidence that the autonomic nervous system is also affected in this disease (1).

Objectives: The purpose of this study was to investigate autonomic cardiac control in patients with ALS.

Methods: Thirty one patients with probable or definite ALS according to El Escorial criteria (17 women and 14 men; average age 57.4 ± 10.5) were compared to 10 healthy controls (6 women and 4 men; average age 42.1 ± 8.4). Patients with previous history of cardiac disease and impaired respiratory function were excluded from the study. Nineteen patients had limb onset ALS while 12 presented with bulbar onset of the disease. Cardiovascular autonomic reflex tests according to Ewing (2), power spectrum analysis of RR variability (low frequency band – LF, high frequency band – HF, LF/HF index), nonlinear heart rate variability analysis (LFnu-RRI, HFnu-RRI, LF/HF-RRI) and baroreceptor function analysis (total slope mean- slSBR) were performed in all subjects.

Results: ALS patients had a significantly higher score of parasympathetic autonomic dysfunction ($p<0.01$). Responses to Valsalva manoeuvre, deep breathing and standing up were significantly altered in comparison to the control group ($p<0.05$). The overall score of autonomic dysfunction was also significantly higher in ALS patients ($p<0.05$). Ewing score of autonomic dysfunction did not correlate with the duration of the disease ($r=0.141$; $p=0.456$). Spectral power of the low frequency band was significantly decreased in the ALS group ($p<0.05$). Patients with bulbar onset ALS exhibited a significantly higher score of parasympathetic dysfunction than patients with limb onset of the disease ($p<0.05$). Baroreceptor activity was lower in bulbar onset ALS patients compared to limb onset patients (mean slSBR 7.33 vs 11.65) and this difference was nearly significant ($p=0.056$).

Conclusions: The results of this study demonstrated impaired cardiovascular autonomic control in ALS with parasympathetic dysfunction and sympathetic hyperactivity. These disturbances occurred both in early and advanced stages of disease. Bulbar onset is associated with marked parasympathetic cardiac impairment suggesting that the pathological process affects both motor and parasympathetic neurons in brainstem nuclei simultaneously.

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P74 NATURAL HISTORY OF AMYOTROPHIC LATERAL SCLEROSIS WITH SPASTIC PHENOTYPE

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Background: The clinical picture of Amyotrophic Lateral Sclerosis (ALS) is a stereotypical one, resulting from a combination of symptoms and signs of upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction. In most patients LMN signs usually prevail, (amyotrophic phenotype) but in a minority of patients the clinical pattern is dominated by pyramidal signs (spastic phenotype).

Objectives: The aim of our study was to investigate the clinical features and the long-term follow-up of a group of ALS patients with the spastic phenotype.

Methods: We reviewed the database of 485 sporadic ALS patients, observed in our Neurological Institute over the last 20 years. Patients were included in the spastic phenotype when spastic paraparesis was a predominant feature in the initial or fully developed phase of the disease. Patients with prevailing LMN signs were defined as amyotrophic phenotype.

Results: We observed 378 patients (78%) with amyotrophic phenotype and 107 patients (22%) with spastic ALS. In the amyotrophic phenotype, a slight excess of males was observed at all ages, with a M/F ratio of 1.3:1. In the spastic group we observed 60 males and 47 females with a M/F ratio of 1.27. However a marked male prevalence occurred in young-adult spastic patients aged <40, (M:F=7.3:1), whereas the proportion of females was greater than males in adult spastic patients, M:F ratio being 0.7:1. The age of onset of spastic patients ranged from 22 to 77 years (median 51 years). Kaplan-Mayer analysis showed a median survival of 71 months (95% CI 59.49–82.50) in the spastic group compared with 42 months (95% CI 36.82–47.17) in the amyotrophic group ($p<0.0001$). Using the multivariate Cox model in the 107 patients, after adjusting for survival factors such as age, gender, site of disease onset and clinical phenotype, spastic phenotype and age remained independent predictors of survival.

Conclusions: ALS with spastic phenotype appears to be a distinctive clinical variant characterized by better prognosis with respect to the classical amyotrophic form. The prevalence of males in patients with onset before the age of 40 and of females in those with older onset suggests that genetic or environmental factors linked to sex, may play an important role in the development of spastic ALS.

P75 NATURAL HISTORY OF PRIMARY LATERAL SCLEROSIS: IMPACT OF MINOR DENERVATION ON PROGNOSIS

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Background: Primary lateral sclerosis (PLS) is a progressive neurodegenerative disorder that affects predominantly the corticospinal tracts. It has been proposed that clinically pure PLS should be distinguished from upper motor neuron dominant amyotrophic lateral sclerosis (UMN-D).

Objectives: To characterize patients with clinical diagnosis of PLS from 1990 to 2006.

Methods: Clinical information and EMG studies of patients with the diagnosis of PLS from 1990 to 2006 were reviewed. Patients were classified as clinically pure PLS if there were no abnormalities on EMG, or UMN-D if there were limited signs of minor denervation on any EMG.

Results: Of the 41 patients in the study, the majority (n=35) had the ascending paraplegic variant, while the remaining patients had Mills variant (n=3) or the bulbar onset variant (n=3) of PLS. Among patients with the ascending paraplegic variant, there was a trend that patients with clinically pure PLS (n=13) had younger age of symptomatic onset than patients with UMN-D (n=22; median 44 vs. 54 years, $p=0.12$). Minor signs of denervation developed in 2 patients with pure PLS and disappeared in 3 patients with UMN-D. There was no difference between time to upper extremity involvement (6.5 vs. 7 years), time to gait aid (7 vs. 8 years), or survival to last clinical follow-up (10 vs. 11 years).

Conclusion: Patients with clinically pure PLS had younger age of onset than patients with clinical syndrome of PLS and minor signs of denervation on EMG. The prognosis is similar between the two disorders, and EMG evidence of minor denervation may be transient.

P76 EVALUATION OF PATIENTS WITH LOWER MOTOR NEURON DISORDERS

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Background: It could be difficult to initially distinguish ALS with clinically predominant lower motor neuron (LMN) involvement from alternative diagnoses of LMN disorders. LMN syndromes have been known to positively affect prognosis.

Objective: To evaluate the association of initial isolated LMN signs and the final diagnosis of MND as well as the disease progression in MND cases from the database of the Krakow MND Center.

Material and methods: We retrospectively analysed a group of 89 patients with initially isolated lower motor neuron (LMN) signs at the disease onset selected out of 450 consecutive MND patients who were referred to our center in the years 2003–2006.

Results: Thirty two patients had the flail-arm (FA) or flail-leg (FL) phenotype of ALS (M:F-3:1; age of onset 47.8 yrs; disease duration 41.3 months); 23 had unilateral distal upper extremities LMN presentation of ALS (16 men, 7 women; age of onset 54.8 yrs; disease duration 37.3 months); 11 had Kennedy's syndrome (SBMA; 11 men; age of onset 43.3 yrs; disease duration 15.3 yrs); 7 had progressive muscular atrophy (PMA) (5 men, 2 women; age of onset 51.3 yrs; disease duration 6.7 yrs); 6 had spinal muscular atrophy (SMA) (6 men; age of onset 41.4 yrs; disease duration 16.4 yrs); 5 had post-polio syndrome (3 men, 2 women; age of onset 47.6 yrs; disease duration 9.1 yrs) and 5 had multifocal motor neuronopathy (MMN) (4 men, 1 women; age of onset 43.2 yrs, disease duration 9.1 yrs).
FA and FL cases presented with isolated LMN signs for a longer period of time before they finally developed ALS and had a longer life span. First LMN presentation in each of the diseases were: proximal weakness of the upper limbs in FA; unilateral foot drop in FL; distal weakness of the lower limbs in SBMA; symmetrical proximal upper weakness in PMA; distal and proximal focal weakness in SMA; distal paresis of the lower limbs in PPS and distal asymmetrical weakness in MMN cases.

Conclusions: Isolated involvement of LMN signs at presentation was diagnosed as different types of motor neuron diseases with usually better prognosis compared to ALS cases.

P77 THE 'FLAIL LEG' VARIANT OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis causes motor neurone degeneration that is typically focal at onset, but progresses to involve all 4 limbs, bulbar and respiratory territories. Variants of ALS have been described, that follow a more focal course, such as progressive bulbar palsy, and the flail arm syndrome. A similar focal presentation in the lower limbs is widely recognized by clinicians, but the characteristics of this variant are not described in the literature.

Objectives: To determine the frequency, clinical features and natural history of the 'flail leg' variant of amyotrophic lateral sclerosis.

Methods: We carried out a retrospective case notes analysis of 310 consecutive patients with a diagnosis of motor neurone disease, attending the Sheffield Care and Research Centre for Motor Neurone Disorders between January 2001 and January 2006. Data on key characteristics of the disease at presentation, and on progression of the disease were collated. Patients presenting with a motor neurone disorder affecting the lower limbs at presentation, and progressing to profound, symmetrical wasting and weakness of the legs, prior to the development of functionally significant involvement of other regions were identified. The clinical characteristics of this 'flail leg' presentation were compared to those of other ALS patients presenting with lower limb onset weakness.

Results: 7 out of 310 (2.5%) of ALS patients developed the 'flail leg' variant of ALS. At presentation, there was clinical evidence in all patients, and EMG evidence in 71% of motor neurone degeneration in a second territory, but the progression of disease in these patients differed from that of classical ALS, with development of a profound, symmetrical, flaccid paraparesis, prior to onset of weakness in a second territory. There was a trend towards longer survival in the patients with 'flail leg' variant (median 58.5 months) versus other patients with lower limb onset (median 39 months, ratio 0.67; 95% CI 030 to 1.02; p=0.063).Typical neuropathological changes of ALS were seen on autopsy in two patients.

Discussion: We describe an uncommon focal variant of ALS, which follows a more benign disease course than classical ALS. The recognition and study of more focal variants of disease, such as the flail arm and flail leg variants, and the factors that cause relative vulnerability of different subpopulations of motor neurones may contribute to our understanding of the pathophysiology of progressive neurodegeneration in ALS.

P78 LARYNGOLOGICAL EVALUATION OF THE PRECLINICAL BULBAR SIGNS IN LIMB ONSET ALS PATIENTS

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Background: Laryngological presentation of ALS has been seldom described in the literature, particularly comparison of laryngological abnormalities in bulbar as well as limb onset ALS cases. Some recent studies underscore the importance of laryngological examination in the ALS diagnostic process. The careful laryngological examination can reveal signs of early dysfunction of the vocal cords and disorganisation of the upper part of the digestive system during swallowing in individuals with limb onset ALS only.

Objective: To test the hypothesis that preclinical signs of bulbar dysfunction occur in limb onset ALS individuals,

via assessing vocal cord changes using fiberoscopic and/ or videostroboscopic examination and disorders of tongue, hypopharynx and UES function during swallowing.

Material and methods: Twenty four selective limb onset ALS individuals, diagnosed according to El Escorial Criteria in the Krakow MND Centre of Jagiellonian University, in 2005–2006, were enrolled in the study. None of them presented with any bulbar symptoms or signs in the neurological examination. We detected vocal cord abnormalities using the standard laryngological examination, as well as the fiberoscopic and/or videostroboscopic examination and swallowing disorders using a single-use air–charged catheter manometry system to assess max. pharyngeal pressure, resting and residual pressure and percent relaxation. The laryngological examination was performed in the ENT Department, Medical College Jagiellonian University in Krakow.

Results: The videostroboscopic examination confirmed the fiberoscopic findings in limb onset ALS cases and have in addition revealed: vocal fold bowing, decreased abduction of the true vocal fold and mucus pooling in some cases. The manometry study showed reduction of pharyngeal pressure and time from pharynx to UES in 10/24 patients especially 6 to 9 months after first examination.

Conclusions: Our results support the hypothesis that degeneration of all the motor neurons in ALS may occur simultaneously, with unknown factors determining the different first clinical presentation/ type of disease onset in each patient.

P79 ISOLATED BULBAR AMYOTROPHIC LATERAL SCLEROSIS (IBALS): CLINICAL AND ELECTROPHYSIOLOGICAL FEATURES

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Background: ALS patients present with bulbar, limb, or respiratory muscle weakness as their initial clinical symptoms. ALS patients with bulbar onset symptoms generally have a less favorable clinical prognosis and a shorter survival time. However, there are a small percentage of patients with bulbar onset ALS whose symptoms remain in the bulbar region. These isolated bulbar amyotrophic lateral sclerosis (IBALS) patients have not been well characterized and they may not have as poor a prognosis as other ALS patients.

Objectives: To characterize the clinical and electrophysiological features of patients with IBALS.

Methods: A retrospective chart review from a tertiary ALS clinic from 2001 through 2006. A total of 543 charts of ALS patients were reviewed. Approximately 100 of these patients presented with bulbar onset symptoms. Of these 100 patients, only 20 patients had no extremity weakness on initial examination.

Results: Eight of the 20 patients were identified as IBALS. Their symptoms were confined to the bulbar muscles after being followed in clinic greater than 2 years (duration 2–8 years; mean 3.1 years). Other clinical symptoms exhibited by these patients include: 3/8 had cognitive impairment, 4/8 had impaired smooth pursuit eye movements, and 2/8 had pseudobulbar affect. Electrophysiological features include: 2/8 had fibrillations and positive sharp waves in the tongue, 5/8 had fibrillations and positive sharp waves in the limbs even though no weakness was found and 2/8 did not have fibrillations and positive sharp waves in the limbs.
11 of the 20 who presented with bulbar ALS went on to exhibit clinical progression to their limbs within 1 to 5 years (mean 2.2). One of the 20 was later diagnosed with PLS.

Discussion and conclusions: Some patients with bulbar onset ALS remained clinically restricted to the bulbar region after more than 2 years. We could not predict which patients would fall into this category. It is uncertain if patients who present with isolated bulbar ALS (IBALS) without progression to limbs have a better prognosis.

P80 MADRAS MOTOR NEURON DISEASE (MMND): ITS CLOSENESS TO BROWN-VIALETTO-VAN-LAERE SYNDROME, YET A DISTINCT CLINICAL ENTITY. PROFILE OF 111 PATIENTS FROM SOUTHERN INDIA SEEN OVER 32 YEARS

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Background: MMND is a juvenile motor neuron disease associated with bilateral deafness. MMND variant also has primary optic atrophy.

Objective: To study the clinical and demographic profile of MMND and its variants evaluated over last 32 years at the National Institute of Mental Health & Neurosciences (NIMHANS).

Design: Retrospective study of medical records coded for anterior horn cell disorders.

Results: There were 111 patients. M:F-57:54. Mean age at onset-16.0 $\pm$ 8.0 years (1–39). Mean duration of illness-59.7 $\pm$ 66.3months(3–360). Patients from Karnataka-63(56.8%), AndhraPradesh-26(23.4%), TamilNadu-16(14.4%), Kerala-4(3.6%), UttarPradesh-2(1.8%). Consanguinity seen in 14(12.6%). Predominant symptom at onset: Deafness-56(50.4%), bulbar weakness-13(11.7%), limb weakness and wasting-35(31.5%). MMND group was 83(74.7%), MMND Variant-8/83.

Familial MMND-28(25.2%), FMMND Variant-17/28. The total group: bilateral optic atrophy seen in 25(24.0%), associated reduced vision in 15. Bifacial weakness in 55(49.5%), dysarthria-84(75.7%), hypophonia in 68/84, dysphagia-74(66.7%), bilateral deafness-102(91.9%), tinnitus-18(16.2%), bulbar palsy-79(71.2%), tongue atrophy-80(72.1%). Limb fasciculation–54(48.6%). Upper limb (UL) distal weakness with wasting-91(82.0%), proximal–43(38.7%), asymmetrical in 59. Lower limb (LL) distal weakness in 62(55.8%). Spasticity in LLs-75(67.6%). DTR's exaggerated in ULs-59(58.1%), LLs-96(86.5%). Extensor plantars-67(60.4%). Mild cerebellar gait ataxia-17(15.3%). EMG in 88(79.3%) showed chronic neurogenic process in 77. Pes cavus-15, scoliosis-8. Pure tone audiometry in all showed bilateral moderate to severe hearing impairment. Distortion-Product-Oto-Acoustic-Emission in 33 patients was normal. Brain MRI in 18 was normal. Muscle biopsy in 19 was normal. Prolonged P100 latency in 5/25 MMNDV. Positive family history in 16 of 111 patients and 27 affected members evaluated and FMMNDV in 15/28 as compared to MMNDV(8/83) in total group (p-0.0001).

Conclusion: This is a large series of patients with MMND, with the classical geographic distribution to Southern India. Hitherto known as a sporadic disorder, familial occurrence was observed in 15% in this study. Deafness was an invariable feature and audiology demonstrated bilateral primary auditory neuropathy. The illness appears to have manifestations similar to BVLL syndrome, but continues to be intriguing in its occurrence among the South Indian population.

P81 MACHADO-JOSEPH DISEASE PRESENTING AS MOTOR NEURON DISEASE

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Background: The differential diagnosis of ALS is a large list of conditions which includes: myopathies, neuromuscular junction defects, spinal cord lesion, as foramen magnum tumours, roots and plexus lesion, motor and systemic disorders.

Objectives: To include Machado-Joseph disease in the list of differential diagnosis of ALS.

Methods: We describe a patient who presented with a 2-year history of motor disturbances and widespread fasciculations. On examination, upper motor neuron signs were disclosed. No clinical sign of cerebellar dysfunction was observed. He had no family history of neurological illnesses. Laboratorial and radiological investigations were normal. Respiratory function tests confirmed mild respiratory muscle weakness. EMG showed signs of loss of motor units and very large motor units of reinnervation (10 mV) in limbs and bulbar muscles, as well as in intercostals. A diagnosis of motor neuron disease was considered.
On follow-up for 2 years after this diagnosis the patient showed progressive signs of cerebellar ataxia, with limb dysmetria and gait ataxia.

Results: Genetic study for spinocerebellar ataxia type III identified an expanded CAG allele in MJD1 gene with 69 repetitions and a normal CAG allele with 23 repetitions (normal 14–33).

Discussion and conclusions: As with ALS, Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is a neurodegenerative disease which progresses over time, with death due to respiratory complications and cachexia. It is a rare disease with a high phenotypic variability. The severity of the disease and the age of onset are dependent on the number of CAG repeat units. It has been described as 5 clinical types, which may represent a continuous spectrum of the disease. Type II is the commonest, with an onset between 20 and 45 years and presenting the three cardinal symptoms – cerebellar ataxia, spasticity and abnormal ocular movements. Type I, with the earliest onset (20–30 years), also has dystonia and type III, the Machado type, with an age of onset above 50 years, is characterized by slowly progressive ataxia of gait, dysarthria, motor and sensory distal polyneuropathy and amyotrophy. The two other types (IV and V) have been described recently and include, respectively, clinical features of parkinsonism greatly responsive to L-Dopa and spastic paraparesis, described in Japanese patients. The involvement of the central nervous system is diffuse, in particular the dentatorubric tract is mostly involved, but the pyramidal tract may be affected, particularly at the spinal ventral horns, thus explaining some of the clinical features in MJD, as in the case of the patient here described.
This report implies that MJD should be included in the differential diagnosis of ALS.

P82 COELIAC DISEASE MIMICKING ALS

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Background: The clinical syndrome of progressive hemiparesis is attributed to Charles Karsner Mills (1845–1931) and can be a rare presentation of MND, often with similar progression to cases of PLS. Corticospinal tract MRI hyperintensity is a non-specific finding in up to 20% of ALS cases. The neurological manifestations of coeliac disease are protean, but have not included these combined clinical and radiological features to date.

Case details: A 44-year-old male presented with a six month history of progressive hemiparesis. Examination

revealed a wasted right thigh, spastic right hemiparesis, a right extensor plantar and widespread hyperreflexia. Sensory testing was normal.
Electromyography, including the masseter, revealed widespread active denervation beyond the affected limbs. MRI FLAIR sequences demonstrated strikingly confluent, non-enhancing, hyperintensity of the left motor cortex and descending corticospinal tract.
Cerebrospinal fluid constituents were normal with no oligoclonal bands detected. HIV serology and JC virus PCR were both negative.
A mild microcytic anaemia with low serum iron and folate levels was discovered. Anti-endomysial antibody testing was strongly positive and a subsequent duodenal biopsy consistent with gluten-sensitive enteropathy.

Management and course: Nine months after commencing a gluten-free diet the patient showed resolution of his arm weakness, greatly improved gait and attenuated corticospinal tract hyperintensity on MRI.

Conclusions: Cases of peripheral motor neuropathy, some with EMG features suggestive of MND, have been previously described in association with coeliac disease, but not in the context of a progressive hemiparesis associated with such strikingly selective corticospinal tract involvement on MRI.
The on-going clinical and radiological improvement with a gluten-free diet in this patient suggests that the Mills' phenotype of ALS should be added to the potential neurological manifestations of coeliac disease.

P83 VALIDATION OF THE EL-ESCORIAL DIAGNOSTIC CRITERIA FOR PATIENTS ENROLLED IN POPULATION-BASED REGISTRIES

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Background: The El-Escorial criteria (EEC) are broadly used for the diagnosis of amyotrophic lateral sclerosis (ALS) in clinical practice and research. The validity and reliability of the EEC were tested in different settings with conflicting results. Data are lacking on the validation of the EEC in patients to be included in population-based registries.

Objectives: To assess the validity and reliability of the EEC in a population-based setting.

Methods: 10 members of the EURALS Consortium involved in the registration of patients with newly diagnosed ALS were the study participants. Each participant examined the medical records (or summary sheets) of 40 patients with ALS and 39 individuals with clinical conditions considered in the differential diagnosis. To reproduce the heterogeneity of data sources at time of registration, the records were different in terms of accuracy and completeness. Patients and controls were subjected to diagnostic assessment with the original and the revised (Airlie House). The validity of the EEC was tested by confronting each investigator with the neurologists treating cases and controls (the "gold standard"). The reliability was tested by assessing the agreement among investigators. In both cases, the inter-rater agreement was measured with the kappa statistic.

Results: Kappa indicated a moderate inter-observer agreement (overall kappa 0.65 for the original EEC and 0.59 for the revised EEC). The agreement was unchanged when subgroup analyses were performed based on different types of records. With one exception, kappa values indicated a moderate to good agreement between the raters and the gold standard for the original EEC, while – with few exceptions – they were slightly lower (but still within the same categories) for the revised EEC.

Conclusions: The original and revised EEC are fairly valid and reliable when case ascertainment is accomplished for a population-based registry. The solid background of the investigators may be a reasonable explanation.

P84 EARLY, SLEEP- AND HANDICAP-INDEPENDENT IMPAIRMENT OF TISSULAR OXYGENATION IN ALS

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Background: A mouse, KO for the promoter region of the VEGF gene, has been shown to develop motor neuron disease. Blood flow in neural tissues is significantly impaired in this animal. VEGF plays a role in the development of microvascularisation and the presence of a specific haplotype of VEGF increases the risk of ALS. In an initial study on 62 ALS patients, we found a significant reduction of O2 saturation determined during nocturnal oxymetry.

Objectives: To determine, prospectively, whether abnormal tissue oxygenation exists in ALS.

Methods: During the initial investigations for ALS diagnosis, we prospectively performed nocturnal oxymetry on each new ALS patient recruited in our center. Data from oxymetry (mean O2 saturation and time below 94%) in ALS were compared to patients with other neurological disorders hospitalized because of symptoms suggesting apnea syndrome (they were severely impared patients with MS, PSP and Parkinson's disease). In some ALS cases, we also studied diurnal oxymetry. All the patients with evidence of respiratory failure (more than 5% of time $<88\%$ in oxymetry, or SVC $<70\%$ or hypoxia evidenced by blood gases) were excluded. Data from oxymetry were

correlated to ALS parameters : gender, age of onset, site of onset and handicap (ALSFRS).

Results: There were 306 ALS patients (158 men and 148 women) mean age 63 (range 22–86), and 88 controls (39 men and 49 women), mean age 61 (range 29–80). Mean O2 saturation during the night was 93.9 in ALS and 95 in controls ($p < 0.0001$). Mean % of time below 94% was 54% in ALS vs. 36% in controls ($p < 0.00001$). All subjects had normal SVC and blood gases. When comparing O2 saturation in oxymetry (at the finger) and by blood gases, a gradient was noted in ALS with arterial O2 saturation being 2 points higher than that by oxymetry, while such a gradient did not exist in controls. In 51 ALS patients, oxymetry could also be performed during the day. In these 51 patients, mean O2 saturation was 94% during the day and 93.5% during the night. Similarly, % of time below 94% was 67% vs 60%. None of these oxymetry data could be correlated with ALS parameters such as handicap, age of onset or site of onset.

Discussion and conclusions: We demonstrated a low O2 saturation in ALS during both the night and the day. This low saturation seems to be constitutive as independent from respiratory parameters and ALS criteria. This allows us to show the existence of an arterio-capillar gradient suggesting chronic tissular hypo oxygenation in ALS. Oxymetry could be useful for obtaining precise data on the % of time spent below 94% which appears the most sensitive criteria among those obtained during this exam. Replication of those results is warranted before ascertaining that this parameter could be a surrogate marker for ALS diagnosis. The possibility that abnormal % time below 94% may reflect early respiratory involvement cannot be ruled out to date. However, the arterio-capillar gradient suggests the existence of an abnormal transmembrane diffusion pattern that is unlikely to be influenced by the respiratory involvement seen in ALS.

P85 BIOMARKERS IN ALS PATIENTS STRATIFIED BY HFE GENOTYPE

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Objective: Determine the impact of hemochromatosis (HFE) gene polymorphisms in ALS by measuring biomarkers in plasma, cerebrospinal fluid (CSF), and muscle tissue.

Background: Five independent groups have published the frequencies of *HFE* polymorphisms in ALS patients compared to various control groups. A meta-analysis of these studies shows that possession of at least one *H63D* allele is associated with a 26% increased risk of developing ALS. We hypothesized that biomarkers could be identified that suggest this allele is associated with alterations in mechanisms that mediate oxidative and inflammatory responses and thus provide an enabling environment for the ALS disease.

Methods: ALS patients were defined by revised El Escorial criteria as clinically definite ALS, clinically probable ALS, or clinically probable ALS – laboratory-supported. Quantitative assays were performed on plasma from individuals with ALS with (n=13) or without (n=17) one H63D allele and neurologically normal controls with (n=14) or without (n=15) one H63D allele, CSF from ALS patients with at least one H63D allele (n=8) or wildtype (n=16), and muscle tissue from ALS patients with at least one H63D allele (n=16) or wildtype (n=22). The potential biomarkers evaluated were beta-2 microglobulin (β2M, a biomarker for several diseases which complexes with HFE), pro-hepcidin (an iron regulatory protein), IL-6, MCP-1, VEGF, SOD1, and oxidatively modified proteins.

Results: Pro-hepcidin was 24.5% higher in plasma of ALS patients than controls ($p=0.029$). There was a significant positive correlation between pro-hepcidin and IL-6 in control plasma ($r^2=0.64$) but not in other groups, and this relationship trended toward a negative correlation in controls with one H63D allele. In CSF, β2M levels were 30.3% higher in ALS patients with an H63D allele than those without ($p=0.013$). In muscle tissue, the H63D allele was associated with 40.7% lower SOD1 expression ($p=0.001$).

Discussion and conclusions: There are changes in relationships and expression of inflammatory biomarkers in blood, CSF, and muscle that differentiate those ALS patients with H63D alleles from wildtype. The data support our hypothesis that the H63D allele establishes an enabling environment for triggers that induce ALS by setting up a dysregulation of iron management that may exacerbate inflammatory responses. We propose that intervention studies take into consideration that individuals with ALS and the H63D allele (approximately 30% of ALS patients) may have altered baseline levels of oxidative stress and altered inflammatory responses which will likely impact their response to therapeutic strategies.

P86 ASCORBATE FREE RADICAL IN CSF OF ALS: AN ERP SPECTROSCOPY STUDY

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Background: It is now evident that the pathogenesis of ALS is very complex. At the time when the symptoms and clinical signs of ALS appear, many critical processes including mitochondrial and cytoskeletal function, glutamate metabolism and regulation of both reactive oxygen

and nitrogen species are already disturbed in motor neurons. The measurement of most free radicals is limited by their reactivity, short lifetime and low steady state levels in biological samples. In contrast, the ascorbyl radical, formed from the one-electron oxidation of ascorbic acid, is unusually stable, and can be readily measured with EPR spectrometry.

Objectives: The aim of the study was to measure the amount of ascorbyl radical by EPR spectrometry in CSF of ALS patients and controls and to measure the amount of ascorbyl radical in CSF after exposure with exogenous hydrogen peroxide (*ex vivo*) in both groups.

Methods: Eleven newly diagnosed SALS patients (8 men and 3 women) aged 54.0 $\pm$ 3.3 (mean $\pm$ S.E.M.) years with probable or definite ALS according to El Escorial criteria were included in this study. The control group comprised eleven age- and sex-matched patients with migraine or tension headache. The mean age of the controls was 52.8 $\pm$ 5.1 years. Samples of CSF were obtained at the point of diagnosis and the remainder of the samples was used for this study. None of the SALS patients or the controls included in this study had blood-CSF barrier dysfunction which was evaluated by the use of CSF/serum albumin quotient detected by a standard method.
EPR spectra were recorded using the following settings: modulation amplitude, 2G; modulation frequency, 100 kHz; microwave power, 10 mW. All spectra were recorded using EW software (Scientific Software). Measurements were performed using quartz capillaries in which Teflon tubes with samples were placed. Computer simulations of EPR spectra were performed using the WINEPR SimFonia computer programme (Bruker Analytische Messtechnik GmbH,Germany).All chemicals, especially H_2O_2, were of at least analytical grade.

Results: We registered a siginificantly increased amount of ascorbyl radical in ALS patients in comparison with controls ($p < 0.05$). Provoked stimulation of ascorbyl radical in CSF by exogenous hydrogen peroxide added *ex vivo* produced a further significant increase in ALS patients in comparison with controls ($p < 0.05$).

Conclusion: The results obtained in this study indicate that increased amount of ascorbyl radical could be a marker of increased oxidative stress in ALS. Provoked *ex vivo* production of ascorbyl free radical by hydrogen peroxide could be regarded as simulation of a naturally occurring compensatory phenomenon in motor neurons of ALS patients in an environment with pre-existing marked oxidative stress.

P87 PROTEIN PROFILING OF CSF AND PLASMA FROM ALS AND CONTROL SUBJECTS FOR PROTEIN BIOMARKERS OF ALS

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Background: There are currently no diagnostic tests for ALS. Protein biomarkers for ALS would have multiple utilities, including diagnostics, new insight into pathologic mechanisms or therapeutic targets, stratification of ALS patients into sub-populations, evaluation of disease progression, and monitoring of drug toxicity or efficacy. We previously reported the generation of a protein biomarker panel from the cerebrospinal fluid (CFS) of ALS patients using mass spectrometry based proteomics (1). In this prior study we used CSF from a total of 23 ALS and 31 control subjects for mass spectrometry. Confirmation of the mass spectrometry results using a larger set of CSF samples would further strengthen and validate our initial findings.

Objectives: We performed protein profiling of CSF and matching blood plasma from 50 ALS, 30 healthy control subjects, 40 Alzheimer's disease, and 30 other neurologic disease controls (total of 150 samples). Results were compared to our prior published data and we also determined if specific protein peaks observed in the CSF can also be detected in the plasma of the same subjects.

Methods: Protein profiling of CSF and plasma was performed by surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS). Data was analyzed by Ciphergen Express 3.1 software to identify protein peaks that can be used to distinguish ALS from control groups by classification tree analysis. We compared ALS subjects to each other subject group either alone or in combination with the other subject groups. We also analyzed the mass spectrometry data using a learning algorithm called Rule Learner (RL) to generate a panel of potential biomarkers that distinguish ALS from control subjects.

Results: We identified specific protein peaks that distinguish ALS from control subjects in both the CSF and blood plasma. Specific proteins altered in the CSF of ALS patients included transthyretin, cystatin C, neuroendocrine protein 7B2, validating our published reports. We also detected mass peaks consistent with VGF, chromagranin B, and osteopondin that were significantly altered in ALS subjects. Specific protein peaks in the plasma could differentiate ALS from control subjects, and we are currently determining if any CSF based proteins are also present in the plasma.

Discussion and conclusions: We have confirmed our prior published results using a larger sample size and suggest that mass spectrometry based proteomics can identify a panel of biomarkers specific to ALS.

Reference:

1. Ranganathan S., Williams E., Ganchev P et al J Neurochem 2005; 95: 1461–1471.

P88 PROTEOMIC ANALYSIS OF NITRATED PROTEINS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH SPORADIC ALS

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Background: Increased production of peroxynitrite, a highly reactive product of nitric oxide (NO), may cause nitration of tyrosine residues of different proteins affecting their functions and leading to their irreversible damage. 3-Nitrotyrosine (3-NT) is a marker of such oxidative damage and increased levels of free or protein-bound NT have been observed in the CNS affected by neurodegenerative diseases including ALS. Increased levels of 3-NT have also been found in the spinal cord of mouse models of familial ALS already at the presymptomatic stage of the disease. Moreover, using a proteomic approach, specific over-nitrated proteins have been identified which may be potentially involved in the mechanisms underlying motor neuron death.

Objectives: In an attempt to identify specific-candidate markers to be used for an early diagnosis and / or as a prognostic factor of the disease, in this study we aimed to examine whether abnormalities in protein nitration levels and specific over-nitrated proteins could be detectable in the peripheral blood mononuclear cells (PBMC) of patients with sporadic ALS.

Methods: We examined levels of NT-bound proteins from PBMC of 15 sporadic ALS patients, compared to 15 healthy controls. Nitrated proteins in PBMC of sALS and controls were separated on two-dimensional (2D) gel electrophoresis, transferred onto low-fluorescence PVDF membrane and probed with anti-NT antibody. NT levels were measured for the single spot using Qdot nanocrystal fluorescence technology. Proteins were identified by MALDI-TOF mass spectrometry on a parallel 2D gel.

Results: Few nitrated proteins were detectable in the two dimensional immunoblot, and some of the protein spots were remarkably increased in sALS patients as compared to healthy controls. They include two major nitrated proteins such as actin and ATP synthase which were also found overnitrated in the spinal cord of presymptomatic familial ALS mouse models. The effect was not related to the severity of the disease as it was observed in patients with either milder or severe impairment. The identification and quantification of the other over-nitrated proteins are in progress.

Conclusions: These data represent the first evidence of increased protein nitration detectable in the PBMC of ALS patients that involves proteins also found in the vulnerable CNS regions from a mouse model of the disease. This analysis may therefore provide a valuable method to identify proteins which could potentially be used as peripheral biomarkers for the diagnosis of ALS and become possible targets for therapies. *Study supported by Ministero della Salute - RF Malattie Neurodegenerative and Fondazione Cariplo.*

P89 MODULATION OF PROTEOLYTIC ACTIVITIES BY CEREBROSPINAL FLUID OF NORMAL SUBJECTS AND PATIENTS WITH NEURODEGENERATIVE AND NEUROINFLAMMATORY DISEASES

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Background: Calpain, caspase-3, cathepsin B and other proteases are involved in both normal cell functioning and in executing cell death programs. Expression of these proteases is changed in the central nervous system of patients with neurodegenerative and neuroinflammatory diseases. Activities of proteolytic enzymes are regulated by endogenous activators and inhibitors, their role in neurologic diseases having been demonstrated. Thus, measuring proteolytic activities to search for their regulators in cerebrospinal fluid (CSF) of patients with different neurological conditions may be of both diagnostic and pathogenetic significance.

Objectives: The aim of our study was to assess effects of CSF of normal subjects and patients with amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) on selected proteolytic activities.

Methods: Sixteen patients with ALS, 10 patients with relapsing-remitting MS and 8 control subjects were included into the study. Effects of CSF on calpain, caspase-3 and cathepsin B activities were measured using fluorimetric assays based on the specific hydrolysis of Ac-LY-AMC, Ac-DEVD-AMC, Z-RR-AMC respectively by purified human calpain I, recombinant human caspase-3, and purified bovine cathepsin B.

Results and discussion: CSF from all subjects studied activated recombinant caspase-3, while it inhibited purified calpain I and cathepsin B. Since CSF of normal subjects contains inhibitors of calpain I and cathepsin B and activators of caspase-3, these substances, though not

fully identified yet, may be involved in physiological modulation of protease activities. The inhibition of calpain I was significantly increased in ALS patients but not in MS patients. The degree of calpain I inhibition correlated with lactate dehydrogenase activity (measure of cell death) and protein level in CSF of patients with ALS but not of control subjects or patients with MS. It can be suggested that accumulation of calpain inhibitor(s) occurs during cell death in the central nervous system of patients with ALS, this process being specific for ALS-related neurodegeneration.

P90 RILUZOLE TREATMENT DOES NOT AFFECT IMPAIRED GROWTH HORMONE SECRETION IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in human adults, characterized by selective and progressive upper and lower motor neuron degeneration in spinal cord, brainstem and motor cortex. The main currently available drug for ALS therapy is riluzole, an anti-glutamatergic compound. Growth hormone (GH) secretion has been recently reported as impaired in most untreated ALS patients (PALS).

Objectives: To investigate whether riluzole treatment might interfere with GH secretion in PALS.

Methods: Ten PALS, 6 men and 4 women (mean age: 59 $\pm$ 11 years) were studied by performing GH-releasing hormone plus arginine test, before and 3 months after starting riluzole (50 mg bid). Blood samples for GH were collected at baseline, and after 30 and 60 min.

Results: Both before and during riluzole treatment, 4 PALS showed severe, 3 PALS mild GH deficiency, and 3 PALS had a normal GH response. Mean peak GH concentrations were similar before and during riluzole therapy (13.4 $\pm$ 10.3 vs 14.2 $\pm$ 10.1 ng/ml; p=NS). No significant correlation was observed between GH levels and age, BMI, disease duration, severity or clinical form (bulbar vs spinal).

Conclusions: Our data point out that GH secretion is reduced in PALS, and riluzole treatment does not interfere with GH secretion. Therefore, evaluation of GH secretion in PALS can also be performed without withdrawing riluzole.

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THEME 4 HUMAN CELL BIOLOGY AND PATHOLOGY

P91 BLOOD MONOCYTE/MACROPHAGE ACTIVATION MARKERS CD68, PCNA AND IL-10 REFLECT DISEASE STATUS IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS)

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Background: CD68, a tissue macrophage marker, is variably expressed throughout monocyte differentiation. Recent autopsy studies of ALS patients showed increased levels of monocytic/macrophage/microglial cell related transcripts in spinal cord in conjunction with activated CD68+ microglia/macrophage cells in close proximity to motor neurons. ALS mSOD1 mouse spinal cords begin to accumulate $CD68^+$ microglia/macrophages early in life, which increases with age and disease severity. Our previous study on blood specimens found elevated levels of abnormally activated monocyte/macrophages (MO) in ALS patients. The current study was performed to extend the earlier observations and evaluate ALS patient blood for markers of pathogenic activation.

Objectives: 1) To test whether activated macrophages expressing CD68 would be present in blood of ALS patients, 2) to evaluate expression of activation marker proliferating cell nuclear antigen (PCNA) and IL-10, (inhibitor of monocyte/macrophage activation), on circulating monocytes in ALS patients and normal controls, and 3) to determine whether blood macrophage activation state in ALS would correlate with clinical stage of disease.

Methods: Flow cytometry was performed to determine the expression of blood monocyte activation markers in heparinized blood samples from 47 sALS patients and 22 normal controls (N). Results from immune studies were evaluated in light of the severity of neurological impairment as determined by the revised ALS Functional Rating Scale (ALSFRS-R).

Results: Patients with sALS had significantly elevated blood MO CD68 and PCNA compared to controls (MeanCD14+CD68+: N, 526 ± 146; sALS, 699 ± 208, $p=0.0010$; MeanCD14+PCNA+: N, 635 ± 358; sALS, 1042 ± 475, $p=0.0007$). There was a negative correlation between levels of CD68 on sALS CD14 cells and disease status as measured by ALSFRS-R (Pearson $r=-0.3425$, $p=0.0198$). Levels of MO PCNA expression were directly related to MO activation status defined by CD14 co-expression of HLA-DR (Pearson $r=0.4753$, $p=0.0007$), and persistent at all levels of sALS disease severity. No significant differences in levels of MO IL-10 expression were observed between sALS patients and controls. However, there was a significant negative relationship between the rate of sALS disease progression (ALSFRS-R/month) and levels of CD14 cell IL-10 expression in sALS blood (Pearson $r=-0.3069$, $p=0.0359$).

Conclusions: This study, for the first time, demonstrates that levels of blood MO CD68 expression, (a marker of disease progression in ALS spinal cords), are elevated and associated with clinical stage of disease in sALS patients. Patients with sALS show evidence for persistent disease-associated monocyte/macrophage activation which may influence and/or induce the faster disease progression related in part to loss of normal MO IL-10 levels. The coupling of persistent MO activation with loss of potential IL-10 regulation on that activation suggests that new therapies targeting activated MOs may be useful in treatment of ALS, and that blood biomarkers levels reflecting MO activation states could be used to monitor response to therapy.

P92 INTERLEUKINS AND TUMOR NECROSIS FACTOR IN SUPERNATANTS OF AMYOTROPHIC LATERAL SCLEROSIS PERIPHERAL BLOOD LYMPHOCYTES

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Background: Imbalance between T_{h1}-type cytokines (Interleukin(IL)-2, IL-12, and Tumor Necrosis Factor(TNF)-α), promoting cell-mediated immune responses, and T_{h2}-type cytokines (IL-6, and IL-10), modulating humoral responses, might lead to altered immune regulation and apoptosis modulation in neurodegenerative disease.

Objectives: The aim of the present study was to assay such molecules in supernatants of peripheral blood lymphocytes (PBLs) from patients with Amyotrophic Lateral Sclerosis (PALS).

Methods: Repeatedly overtime blood samples from PALS were drawn in the morning, and plasma was stored immediately at $-20^{\circ}C$. Freshly isolated PBLs were cultured for three days. Plasma and PBL supernatant concentrations of IL-2, soluble IL-2 receptor (sIL-2R), IL-6, soluble IL-6 receptor (sIL-6R), IL-10, IL-12, TNF-α, sTNF-RI, sTNF-RII were quantified by enzyme-linked immunoadsorbent assay (ELISA).

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Results: Reduced amounts of secreted cytokines in PBL supernatants were found as compared to cytokine levels in plasmas from PALS. In particular, more reduced amounts of TNF-α and IL-12 than those of IL-6 and IL-10 were assayed.

Conclusion: Our data of significantly decreased amounts of T_{h1}-type cytokines in PBL supernatants from PALS as compared to cytokine levels in plasmas might depend on different "*in vitro*" vs "*in vivo*" micro-environments, so that "*in vivo*" PBLs are conditioned to produce more T_{h1}-type cytokines, as they were in a "more toxogenic" environment. Such findings, therefore, might support the concept of a systemic immunobiochemical derangement in PALS.

P93 FIBROBLAST GROWTH FACTOR AND INSULIN-LIKE GROWTH FACTOR LEVELS IN SERA FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Basic Fibroblast Growth Factor (bFGF) and Insulin-like Growth Factor-1 (IGF-1) are trophic factors for motor neurons and glia. In neurodegenerative disease, unbalance between neurotrophic and neurotoxic factors ultimately causes motor neuron cell death (by a direct effect and/or by activating gliocytes which in turn might initiate apoptotic pathways in motor neurons themselves).

Objectives: To assay bFGF and IGF-1 in sera from Amyotrophic Lateral Sclerosis (ALS) patients.

Methods: We assayed repeatedly over a two-year period bFGF and IGF-1 in sera from 57 ALS patients (24 women and 33 men; mean age $\pm$ SD: 64 ± 12 yrs). Disease severity was scored by means of the ALS Functional Rating Scale, and patients subgrouped accordingly into 3 classes: I (scores between 40 and 31), II (scores from 30 to 11), and III (between 10 and 0). Blood samples were drawn in the morning, and serum was stored immediately at -20°C. Growth factors were quantified by enzyme-linked immunoadsorbent assay.

Results: Growth factors data concern assays at time of diagnosis (t_0) and those at time of the most recent clinical examination (t_n). Mean bFGF levels were lower in class I and class II patients at t_n vs t_0 (1.1 ± 0.9 vs 2.7 ± 1.8 pg/ml, and 3.6 ± 4.1 vs 5.9 ± 8.4 pg/ml, respectively). Mean bFGF levels were higher in class II vs class III patients at t_n vs t_o.
Mean IGF-1 levels were significantly ($p < 0.05$) higher, in both class I and class II patients, at t_n vs t_0 (133.7 ± 68.3 vs 43.3 ± 21.2 ng/ml, and 288.9 ± 430.8 vs 88.1 ± 125,3 ng/ml, respectively). Mean IGF-1 levels were higher in class I vs class II and class II vs class III patients at t_n vs t_0.

Discussion: Although overtime changes of serum bFGF are not univocal and are unrelated either to progression or severity of disease, serum IGF-1 values appear to be directly linked to biological responses to neurotoxic noxae, as if a milder disease state maintained for a longer period of time is associated with increased IGF-1 production.

P94 IS ANGIOGENIC FACTOR REGULATION IMPAIRMENT RESPONSIBLE FOR MOTONEURON DEGENERATION IN ALS?

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Background: Evidence for a lack of up-regulation of vascular endothelial growth factor (VEGF) is suspected in hypoxemic sporadic ALS patients. Angiogenic factor regulation is poorly studied in ALS. First studies of CSF $VEGF_{165}$, EPO and Angiopoietin showed significantly lower levels in hypoxemic ALS patients.

Objective: To specify systemic deregulation of hypoxemia mechanisms in ALS by studying monocytes and their $VEGF_{165}$, PGE-2 and angiopioetine-2 excretion capacities in normoxic vs hypoxic conditions.

Method: Monocytes were extracted and exposed in culture chambers in 3 conditions: normoxia, acute hypoxia (1 hour) and prolonged hypoxia (24 h). Two conditions were studied: baseline and in addition of PGE-2 (1.10^{-6}). We measured $VEGF_{165}$, PGE-2 levels and their RNA levels in each condition.

Subjects: Blood peripheral cells of 15 patients at an early stage of sporadic ALS without respiratory insufficiency were compared to 15 healthy age matched controls.

Results: At baseline in normoxia, we observed no significant difference in VEGF levels and RNA between ALS and controls. In hypoxic conditions $VEGF_{165}$ levels were significantly decreased by 50 % in ALS patients compared to controls. Maximum decrease by 71 % in $VEGF_{165}$ synthesis was observed in addition of PGE-2 during acute hypoxia.

Discussion: Our data suggested that functional abnormalities in the regulation of angiogenic factors (PGE-2/ $VEGF_{165}$ and Angiopoïetin-2/ $VEGF_{165}$) during hypoxia could be implicated in motorneuron degeneration. This could be relevant during ALS progression since acute intermittent hypoxia frequently occurs particularly due to diaphragmatic failure.

P95 EFFECTS OF VITAMIN AND RILUZOLE TREATMENT ON LEVELS OF REACTIVE OXYGEN SPECIES, SOD1 ACTIVITY AND TOTAL ANTIOXIDANTS IN PERIPHERAL TISSUES OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: In a previous work (1) we reported reduced levels of reactive oxygen species (ROS) in peripheral blood and SOD1 activity in erythrocytes of patients affected by sporadic amyotrophic lateral sclerosis (sALS). Moreover, lower expression of intracellular SOD1 was observed in sALS lymphocytes (2). The only drugs approved by the FDA for ALS treatment are vitamins and riluzole. Despite the evidence of increasing presence of oxidative stress markers, therapeutical trials with antioxidants, such as vitamin E and C, have shown scant benefit. Riluzole has been reported to extend survival of ALS patients by three months.

Objectives: The aim of this work was to assay overtime in sALS patients the effect of vitamin and riluzole treatment on plasmatic ROS levels, SOD1 activity and plasmatic total antioxidant proteins (TAO). The intracellular SOD1 expression was also assayed before and during vitamin and riluzole administration.

Methods: Erythrocyte SOD1 activity, plasmatic ROS and TAO levels were assayed in 79 sALS patients before treatment and after vitamin and/or riluzole administration every three months. SOD1 activity was assayed by its ability to inhibit superoxide radical-dependent reactions, and expressed as U/g of haemoglobin. ROS and TAO levels were evaluated by colorimetric methods (Diacron International and Randox Laboratories, Italy), and expressed as U CARR and mmol/l, respectively. Intracellular SOD1 expression was evaluated by western blotting in lymphocytes from 10 sALS patients at the time of diagnosis and after vitamin and/or riluzole administration.

Results: Mean SOD1 activity values in sALS patients were lower than reference values, and were not modified by vitamin and/or riluzole treatment. Mean plasma ROS levels were under normal range and significantly increased ($p < 0.05$, ANOVA) in consecutive blood drawings. TAO amounts were significantly lower ($p < 0.05$, Student's *t*-test) compared to normal range values and remained almost unchanged after vitamins and/or drug administration. Intracellular SOD1 expression significantly ($p < 0.05$, Student's *t*- test) decreased during ALS progression.

Discussion: SOD1 activity does not seem to be involved in increased oxidative stress, while evidence suggests that changes of intracellular SOD1 expression may play a role. Pharmacological treatment does not modify increased plasmatic ROS during disease progression and lower levels of plasmatic TAO. Our data show that vitamin/riluzole administration does not modify oxidative stress markers, at least in peripheral tissues.

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P96 CSF DETECTION OF ENTEROVIRUS GENOME IN ALS: A STUDY OF 240 PATIENTS

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Background: Persistent infection caused by an enterovirus (EV) has been considered as a potential causal factor for ALS development because of the similarity with the post-poliomyelitis syndrome. Recent studies showed contradictory results in assessing a relationship.

Objectives: We implemented from 1997 to 2002 a multicentric study aimed at detecting by RT-PCR, EV RNA in the CSF from ALS patients and controls.

Methods: CSF specimens were collected from ALS suspected patients according to revised El Escorial criteria, admitted for hospitalisation in one of the 7 participating ALS centres. Control samples were obtained from NeuroBiotec-Banques of Lyon (France). In 2007, all the ALS patients were verified and classified as confirmed ALS, differential diagnosis or non validated cases. RNA was extracted using Tri-reagent method (Sigma Aldrich, France). Each RNA pellet was reconstituted for reverse transcription. cDNA was then subjected to PCR primers that recognize the 5' UTR of all known EV and to electrophoresis in agarose gel to observe a band of 154 bp consistent with EV. Student t-test analysis was used to compare the number of positive RT-PCR between the groups.

Results: We collected 306 CSF ALS samples. 240 patients were confirmed ALS, 51 had differential diagnosis, 15

were non validated cases. We obtained 318 CSF samples of controls. In the ALS group, we recorded age, sex, clinical form, diagnosis delay, evolution duration, and area of birth, year of diagnosis and year of sample for further multivariate analysis. Epidemiologic and clinical criteria of ALS patients were compatible with the general ALS population data. Thirty-five out of the 240 ALS patients (14.6%) and 26 of the 318 control patients (8.8%) tested positive for EV. This difference was statistically significant ($p < 0.05$).

Discussion: The present study reveals statistical evidence of a relationship between the presence of EV sequences in CSF and ALS disease. It seems however that 8.8% of positive results in the CSF controls are a slightly high result. EV are among the most common of human viruses and most infections are unapparent. Their tropism for the CNS could explain their detection in the controls. Chronic EV infection was developed in a human glial precursor cell line and was associated with an aberrant splicing of the high affinity glutamate transporter EAAT2 pre-messenger RNA and a significant loss of EAAT2 protein expression. This could be the putative link between EV infection and the glutamate-mediated excitotoxicity for motor neurons, similar to that observed in patients with ALS.

Conclusion: In the light of these results it is not possible to predict a patient's susceptibility to developing ALS by testing CSF samples for EV. However, combined detection of EV in spinal cord and CSF samples of each patient could not be performed and would have increased positive results in ALS patients. Further works are required to confirm that the virus we detected was persistent and had a pathogenic role, and to determine how this persistent infection has occurred.

P97 INCLUSIONS CONTAINING SUPEROXIDE DISMUTASE-1 ARE REGULARLY PRESENT IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS LACKING MUTATIONS IN THE ENZYME

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Background: Six percent of ALS is linked to mutations in the ubiquitously expressed enzyme superoxide dismutase-1 (SOD1), making it the most common cause of the disease. The cause of the remaining cases is largely unknown. Histopathologically, three types of inclusions are seen in neurons of the brain and spinal cord – Bunina bodies, Lewybody-like-hyaline inclusions and skein inclusions. In addition, in familial ALS caused by mutated SOD1 another type of inclusions are observed, which contain aggregated units of the misfolded enzyme.
Misfolded and aggregation-prone forms of mutant SOD1s are thought to trigger the disease.

Objectives: The aim of this study was to investigate if SOD1 is involved in ALS patients lacking mutations in the enzyme and to find the subcellular localisation of the SOD1 inclusions.

Methods: CNS tissue was collected from 30 patients with sporadic ALS (SALS) and 7 patients with familial ALS (FALS), from 27 control patients with other neurodegenerative diseases and 19 control patients without neurological disease. Paraffin embedded spinal cord sections were immunostained using immunohistochemistry and immunofluorescence for detection and co-localization of SOD1, lysosomes, endoplasmic reticulum and mitochondria. Three peptide antibodies corresponding to amino acids 4–20, 57–72 and 131–153 in the human SOD1 sequence were used as primary antibodies for detecting SOD1 inclusions. For recognition of organelles the following antibodies were used: cathepsin D for lysosomes, KDEL for endoplasmic reticulum, HSP70mito for mitochondria and an antibody recognizing the human TAR DNA-binding protein (TDP-43). Confocal microscopy was used for analysis of the samples.

Results: Numerous small somal round inclusions in motoneurons, immunoreactive for SOD1, were a feature of both sporadic and familial ALS. They were relatively homogenous in size and measured 0.5–3μm.
All investigated SALS and FALS patients had SOD1 containing inclusions. In contrast none or very few controls had inclusions.
Double immunofluorescence staining shows a partial colocalization with the lysosomal marker cathepsin D. No colocalization was found between SOD1 inclusions and endoplasmatic reticulum, mitochondria or TDP-43.

Conclusions: Inclusions of misfolded, aggregated SOD1 are a general histopathological feature, regularly present in the motoneurons of ALS patients lacking mutations in the enzyme. The inclusions are situated in the cytoplasm of the neurons and do not co-localize with mitochondria, endoplasmic reticulum or TDP-43 and only partly with lysosomes. Our results suggest that SOD1 is involved in the pathogenesis of all forms of ALS.

P98 TDP-43 IMMUNOREACTIVITY IN FAMILIAL ALS WITH OR WITHOUT SOD1 MUTATIONS, GUAM ALS, AND SPORADIC ALS

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Background: TAR DNA-binding protein (TDP-43) was identified as a component of ubiquitin-immunoreactive (-IR) inclusions in sporadic ALS and frontotemporal lobar

degeneration with ubiquitin-only-IR inclusions (1,2). TDP-43 was also reported to be expressed in non-SOD1 familial ALS cases, but not in the cases with SOD1 mutations (3,4).

Objectives: The aim of this study was to investigate whether abnormal cytoplasmic TDP-43-IR inclusions occurred in familial ALS caused by different SOD1 mutations, Guam ALS, as well as sporadic ALS cases.

Methods: Formalin fixed paraffin-embedded spinal cord serial sections were obtained from 13 sporadic [68.8 ± 2.7 (mean age ± SEM)], 6 familial ALS (4 with SOD1 mutations: I113T, D101G, L8V, H48Q) [50.0 ± 3.8] and 2 Guam [58.5 ± 4.5] ALS. Sections were immunohistochemically stained with mouse monoclonal antibodies to p62 Lck Ligand, phosphorylated tau (clone [AT8]), phosphorylated neurofilament (clone [2F11]), alpha-synuclein (clone [KM51]), and rabbit polyclonal antibodies to TDP43, ubiquitin, cystatin C.

Results: In the case with H48Q SOD1 mutation, there was a single TDP-43-IR neuronal inclusion, which co-localised with p62. The D101G case showed ubiquitin and p62-IR neuronal and glial inclusions, which were negative for TDP-43. The L8V case had frequent p62-IR glial inclusions, but no TDP-43 positivity. Of the two non-SOD1 familial ALS cases, one showed TDP-43-IR neuronal inclusions, which were ubiquitin and/or p62 positive. All sporadic ALS cases had TDP-43-IR neuronal inclusions. These were positive for ubiquitin and p62. Except one case, all sporadic cases showed p62 and TDP-43 glial inclusions. None of our sporadic or familial ALS cases stained with phosphorylated tau, phosphorylayed neurofilament or alpha-synuclein. In both Guam ALS cases, there were ubiquitin and p62-IR neuronal inclusions, which were also positive for TDP-43. The vast majority of TDP-43 positive neurones were negative for phosphorylated tau, but one anterior horn neurone in one Guam case appeared to co-express phosphorylated tau and TDP-43.

Discussion and conclusions: These findings suggest that TDP-43-IR neuronal and glial inclusions are consistently present in both sporadic and Guam ALS, but not in non-SOD1 and SOD1 familial ALS. Therefore, TDP-43 seems to be a reliable marker for the neuronal and glial inclusions present in sporadic and Guam ALS.

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P99 IMMUNOHISTOCHEMICAL STUDY ON TDP-43 PROTEIN IN AMYOTROPHIC LATERAL SCLEROSIS/PARKINSONISM-DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Background: Anti-TDP-43 antibody positive inclusion was reported in the brains and spinal cords of frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusion (FTLD-U) and sporadic ALS in 2006. The purpose of this study is to reveal TDP-43 protein in Kii ALS/PDC brains and spinal cords immunohistochemically.

Method: Formalin-fixed, paraffin-embedded sections of the medial temporal lobes and spinal cords derived from six patients with Kii ALS/PDC were submitted for the study. Anti-tau antibody (AT8, Inogenetics) and anti-TDP-43 antibody (TAR DNA-binding protein 43, Protein Tech Group) were used for immunohistochemical analysis.

Result: Immunohistochemistry with anti-TDP-43 antibody revealed neuronal cytoplasmic, dot-like or threads structures in the hippocampus and spinal cord. Some of the TDP-43 positive inclusions co-existed with tau positive inclusions in the double staining with anti-tau and anti-TDP-43 antibodies.

Conclusion: Recently TDP-43 was reported to deposite in the Gaum PDC brains. Accumulation of TDP-43 may be a common process in Guam and Kii ALS/PDC.

P100 EXPRESSION AND CHARACTERIZATION OF A NOVEL PROTEIN (FLJ10986) ASSOCIATED WITH SPORADIC ALS

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Background: In a recent study the whole genome was screened for genetic polymorphisms (SNP) associated with sporadic amyotrophic lateral sclerosis (SALS) using blood samples from 1,152 SALS patients and 1,450 controls.

Approximately 40 genes were found to be associated with SALS. The top-hit was a gene called FLJ10986 located on chromosome 1 with unknown function.

Objectives: The cell types that express FLJ10986 within the nervous system are unknown, and the functional role of this protein is unclear. The objective of the present study was to further characterize its expression and distribution in the central nervous system (CNS).

Methods: All tissue samples were genotyped for the presence of FLJ10986 polymorphisms. Spinal cord tissue homogenates from 10 SALS cases and 6 control cases, and cerebrospinal fluid (CSF) samples from 6 SALS and 4 control subjects were analyzed by Western Blot (WB). We also analyzed homogenates from fetal brain, liver, intestine, heart, lung, kidney and human serum by WB. For WB samples were electrophoresed on NuPage 12% Bis-Tris gels, proteins were transferred onto nitrocellulose membranes and labeled with primary mouse polyclonal antibody to FLJ10986 protein (Novus Biologicals) at 1:700 concentration. Protein was detected by enhanced chemilluminescence (ECL). Paraffin-embedded lumbar spinal cord sections from 8 control and 17 ALS cases were used for immunohistochemistry. After de-paraffinization and antigen retrieval sections were incubated with the FLJ10986 mouse polyclonal antibody at 1:500 dilution and staining was developed using ABC reagent and NovaRed substrate.

Results: High expression of FLJ10986 protein of approximately 48 kDa was detected in serum and CSF by WB. In spinal cord tissue homogenates two protein bands for FLJ10986 were detected of approximately 45 kDa and 48 kDa. No significant differences in the levels of total FLJ10986 protein could be detected between ALS and control subjects in either CSF or spinal cord tissue homogenates. However, SALS cases containing the FLJ10986 polymorphism identified in the genome wide association screen had increased levels of the higher molecular mass FLJ10986 protein band. Immunohistochemistry showed that FLJ10986 may be expressed in glial cells as well as fiber tracts.

Discussion and conclusions: Our results indicate that the FLJ10986 protein is expressed in two different mass variants (45 kDa and 48 kDa) in the CNS and that one (48 kDa) may be correlated to genetic polymorphisms in SALS. The protein appears to be expressed predominately in glial cells and fiber tracts. Continued studies in cell culture model systems will clarify how FLJ10986 contributes to pathogenic mechanisms related to ALS.

P101 NRF-ARE SIGNALING PATHWAY IN POST MORTEM TISSUE OF AMYOTROPHIC LATERALS SCLEROSIS (ALS) PATIENTS: AN *IN SITU* HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY STUDY

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Background: Oxidative stress and inflammation are important pathomechanisms in Amyotrophic Lateral Sclerosis (ALS). The aim of the present study is to investigate the role of the Nrf-ARE signaling pathway in the ALS-related selective degeneration of motor neurons. Nrf (Nuclear erythroid 2 related factor; Nuclear respiratory factor) 1 and – with higher potency - Nrf2 are basic region leucin-zipper transcription factors. After activation and translocation to the nucleus, they bind to the antioxidant response element (ARE), a regulatory enhancer region within gene promoters. This regulates the expression of more than 200 genes involved in the cellular antioxidant and anti-inflammatory defense. Among them are classical phase 2 detoxification enzymes such as NAD(P)H quinone oxireductase and glutathione, enzymes which are necessary for glutathione biosynthesis, extracellular superoxide dismutase, glutamate-6-phosphate-dehydrogenase, heat shock proteins and ferritin, furthermore pro- und antiinflammatory enzymes such as cyclooxigenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and heme oxigenase-1 (HO-1) (1,2).

Objectives and methods: In the present study, we investigated the mRNA and protein distribution of Nrf1, Nrf2, the endogenous Nrf2-inhibitor Keap1 and the transcriptional co-activator PGC-1α (peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α) in post mortem primary motor cortex of ALS-patients and control tissues at the cellular level using *in situ* hybridization and immunohistochemistry to find out whether disease-specific alterations of the elements of this pathway occur in ALS.

Results: In the film autoradiograms from the ISH-experiments, we observed a weaker mRNA-signal for Nrf1 than for Nrf2 and PGC-1α without obvious differences of the cortical mRNA expression. By immunohistochemistry, we found reduced expression of Nrf1, Nrf2 and PGC-1α in the ALS patients with a different intracellular staining pattern for Nrf1 and 2: they showed a more pronounced extranuclear localization in the controls than in the ALS-tissue. The endogenous inhibitor of Nrf-1 and 2, Keap1, was increased in neurons from ALS patients as compared to controls.

Conclusion: Our histological findings point towards an imbalance in the cellular response to oxidative stress and inflammation at least at the end stage of the disease. These results are in line with a study in a cellular model of familial ALS where a reduction of Nrf-ARE-regulated genes was found (3).
Further studies in tissues from mutant ALS-mice at presymptomatic and symptomatic stages in comparison to wildtype-mice are required to find out whether changes occur early in the disease course. This could further support the hypothesis that the Nrf-ARE pathway represents a novel therapeutic target in neurodegenerative disorders.

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P102 MITOCHONDRIA IN DORSAL ROOT GANGLION CELLS ARE AFFECTED IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Recent studies have found increasing evidence of the involvement of mitochondrial damage in the course of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) and mutant SOD1 transgenic animal models of familial ALS. However, little information is available regarding the morphological changes in the mitochondria in ALS. In particular, mitochondrial changes in dorsal root ganglion cells have not yet been examined.

Objectives: To examine whether or not mitochondria in dorsal root ganglion cells are affected in cases of sporadic ALS.

Methods: We conducted an electron microscopic examination of the mitochondria in dorsal root ganglion cells of lumbar spinal cord in 11 sporadic ALS patients (aged 48–76 years), and 12 age-matched, non-neurological control individuals (aged 49–83 years).

Results: In both the controls and ALS patients, unusual inclusion bodies were frequently observed in the mitochondria in the somata of the ganglion cells examined. The inclusions consisted of a bundle of filamentous structures varying in size and number as seen in longitudinal sections. Such inclusions were frequently present in the inner compartment and/or intermembrane space, often expanding to form large bundles in the dilated intermembrane space. These structures quite frequently protruded towards the outside on one side or both sides and were surrounded by the outer membrane of the mitochondria. On transverse sections, tubular structures measuring approximately 40 nm in diameter were observed. The number of inclusions was significantly higher in the ALS patients than in the controls ($P < 0.0001$) (ANCOVA: Bonferroni method). Regularly spaced transverse processes similar to the rungs of a ladder were occasionally observed in the intermembrane space, along with infrequent but markedly increased cristae and stubby mitochondria.

Conclusions: We concluded that mitochondrial abnormalities may be involved in the degenerative processes in the dorsal root ganglion cells in cases of sporadic ALS; these findings therefore suggest that ALS is a widespread, more generalized disorder than previously thought, and that the degeneration is not confined to the motor neuron system.

P103 MYOCARDIAL INVOLVEMENT IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Background: Spinal and bulbar muscular atrophy (SBMA) is a hereditary neurodegenerative disease caused by an expansion of a trinucleotide CAG repeat encoding the polyglutamine tract in the androgen receptor (AR) gene. SBMA chiefly occurs in adult males, whereas neurological symptoms are rarely detected in females having mutant AR gene. Testosterone-dependent nuclear accumulation of the pathogenic AR protein plays a pivotal role in the pathogenesis of SBMA. The mutant AR is expressed not only in the nervous system but in non-neuronal tissues, providing the molecular basis of systemic complications such as liver dysfunction and diabetes. The causes of death in SBMA include sudden death, suggesting that cardiac involvement is present in this disease.

Objectives: The aim of this study is to elucidate the myocardial pathology and its clinical implication in SBMA.

Methods: Electrocardiograms from a total of 99 SBMA patients were reviewed. Echocardiography and myocardial scintigram were also analyzed. Anti-polyglutamine immunohistochemical analysis was performed on the myocardium of patients and that of SBMA transgenic mice.

Results: Abnormal electrocardiogram findings were detected in 49 out of 99 patients. The most frequent finding was ST-T abnormality in V5,6 (23.2%), followed by ST elevation in V1-3 with right bundle branch block (17.2%), left ventricular hypertrophy (4.0%), light bundle branch block (4.0%), and QT elongation (2.0%). ST elevation in V1-3 was often accompanied by J wave.

No patients showed abnormality in echocardiography or in myocardial scintigram. In one patient with ST elevation in V1-3, ventricular fibrillation was evoked in the electrophysiologic study, and an implantable cardioverter defibrillator was implanted. Routine HE staining of myocardium showed no sign of degeneration. Immunohistochemistry using a specific antibody against extended polyglutamine demonstrated nuclear accumulation of mutant AR protein in the myocardium of patients and in that of model mice.

Discussion and conclusions: The present study indicates that the myocardium is frequently affected in SBMA. Arrhythmia might trigger sudden death in the advanced case of SBMA. Almost half of the patients demonstrated arrhythmic changes in the electrocardiogram, whereas structural abnormality was not detected in this study. In addition to hypertensive change, several electrocardiographic abnormalities irrelevant to high blood pressure were found. Immunohistochemical analysis suggests that mutant AR, the causative protein of SBMA, possibly plays a role in the electrophysiological complication in myocardium. Taken together, it is likely that at least some features of myocardial complication are disease-specific pathological change in SBMA.

P104 ALTERATIONS OF THE RETINOIC ACID SIGNALING PATHWAY IN ALS

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Previous reports have shown that transthyretin (TTR) protein levels are decreased in the cerebrospinal fluid of amyotrophic lateral sclerosis (ALS) patients and may represent a protein biomarker for ALS. TTR plays multiple physiological roles and functions within the retinol signaling pathway important for modulating gene expression, protein aggregation and antioxidant activity for maintenance of cell survival. In our current study, we examined multiple proteins that function within this retinoic acid signaling pathway using immunoblot and immunohistochemistry of tissue samples from ALS and control subjects. Immunostaining for cytoplasmic retinol binding proteins including cellular retinol binding protein (CRBP) and cellular retinoic acid binding proteins (CRABP)-I and II shows altered levels of expression in ALS. CRBP immunoreactivity is significantly lower in abundance in spinal cord motor neurons of ALS patients, and CRABP-I levels are also decreased in ALS subjects. Differences with respect to CRABP–II were also detected, and immunostaining for downstream nuclear receptors including RARα, RARβ and RXRβ were also assessed. Although RARα exhibited no differences in expression or subcellular distribution in ALS patients, RARβ demonstrated increased nuclear immunoreactivity within motor neurons of ALS subjects. RXRβ immunostaining also appears more intense in ALS tissue sections. These results indicate that retinoic acid signalling is altered in ALS patients and may represent a new therapeutic target for ALS. Specifically, differences with respect to particular nuclear receptors may lead to differences in binding at nuclear response elements and, therefore, altered transcriptional regulation. Further studies to evaluate the functional significance of these alterations of the retinoic acid signalling pathway are being pursued in cell culture assays.

P105 THE REDOX DISORDER PELLAGRA - NAD(P)(H)DEFICIENCY - CAUSED A (FORGOTTEN) PREVENTABLE FORM OF MND: A CURRENT LESSON FOR CELL DIFFERENTIATION, MAINTENANCE OF ORDER AND SELECTIVE DEGENERATION?

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Lussana (1856) noted fasciculation of the tongue in pellagrins before Charcot described MND (1). Others noted rare ''exquisite replicas'' of MND. Mixed motor neurone syndromes were commoner and included wasting, fasciculation and cramps (2–4). Pellagra is a deficiency of nicotinamide and tryptophan due to a poor vegetarian diet. These essential xenobiotics produce NAD(P) vital as the chief carrier of hydrogen - the electron donor, reductive and radical power-source. NAD(H) composes ''Turing'' oscillating gradients to instruct circadian and other rhythms key to development (5): and counteracting degeneration consequent on the second law of thermodynamics. Disorders such as the equally varied and avoidable syndromes on Guam are also linked with a poor vegetarian diet and additional redox stresses, perhaps excess selenium/sulphur from volcanism entering the food chain through cycads and redox sensoring Tau and TRPM variants (6).

Redox potential is defined by the ratio of NAD(P) to NAD(P)H and may be the maxwellian ''decider'' on the smart use of energy currents best for the maintenance of order and survival: and ''boss'' the balances between catabolism and a hierarchy of anabolic programmes from division to differentiation (7). Knock on effects for reduced thiols and the ATP/ADP ratio exist. NAD(P)(H) hits cause a downstream oxidative and excitotoxic atmosphere and individualised mitochondrial and transcriptional collapse.

Mutant SOD causes abnormal redox that reduces the organisms ''robustness'' to ward off environmental agents that increase reductive stress (8). Conversely redox alters structure, aggregation, biochemical and electrogenic properties of SOD via cysteine residue oxidation. NADPH levels and its oxidation is involved in MND in relevant locations for influencing selective neuronal death and involves astrocytes and microglia (9). NAD(P)(H) balances are adversely affected by deficient diets but can be caused by ''drowning'' in excessive quantities of

precursors: as well as by caloric intake, toxins and insults to DNA that consume NAD via the use of ribosylating PARPs, ARTs and Sirtuins. Second hits possibly from selenium or sulphur excess, that like nicotinamide are methylated, leads to shortage of the methyl donor SAM gene silencing and reduced production of glutathione; these conspire to affect redox potential and a homeostatic use of autocarnivory to release NAD(H) and methyl groups.

Measurement and reduction of reductive stress by manipulating precursors or enzymes that regulate NAD or NADP should be explored in MND given this lost clinical data and metabolic plausibility. Predictably this involves interacting genotypes and ecotypes (GxGxE) that converge as cell specific nutrient/energy currents that may fail selectively over the long term depending on earlier trade-off arrangements and contemporary circumstances.

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THEME 5 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P106 MAGNETIC RESONANCE SPECTROSCOPY MONITORING OF THE EFFECTS OF MINOCYCLINE TREATMENT ON ALS PATIENTS

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Background: The only FDA-approved treatment for ALS is riluzole, an antiglutamate neuroprotector, which can extend life by no more than 2–3 months. Several other drug candidates are presently under investigation, including the anti-apoptotic agent minocycline (1). Although minocycline shows promise in both animal and human studies, its action on the brain remains to be fully characterized (2). In a number of studies, the non-invasive technique magnetic resonance spectroscopy (MRS) has been used to monitor the effects of ALS on brain metabolites, especially a reduction of the neuronal marker *N*-acetylaspartate (NAA) in the motor cortex (3).

Objectives: The objective of this study is to use MRS to measure brain metabolite variations in ALS patients following the administration of minocycline in order to assess its effects on the disease.

Methods: Ten newly diagnosed ALS patients (62.5 ± 10.3 years) were recruited and submitted to MRS examination before minocycline administration (t=0) and at times 3 and 6 weeks after onset of minocycline treatment (200 mg/day; no other treatment). Single-voxel MRS data were acquired in the precentral gyrus and in the brainstem using the PRESS pulse sequence with TE=30 ms or 135 ms, and TR=1500 ms on a GE 1.5 T MR instrument. Metabolite ratios NAA/creatine (Cr), choline (Cho)/Cr, *myo*-inositol (mI)/Cr, glutamine + glutamate (Glx)/Cr, NAA/mI and NAA/(Cr+Cho) were determined using the LC Model software. Statistical analyses were performed using repeated measures ANOVA.

Results: In the precentral gyrus, none of the metabolite ratios showed significant variations following minocycline administration. Larger variations were measured in the brainstem at t=6 weeks: NAA/Cr +8.7%; Cho/Cr, +15%; mI/Cr, +20%; Glx/Cr, +6.5%; NAA/mI, −11%; NAA/(Cr+Cho), +4.1% but only mI/Cr showed a tendency toward statistical significance (p=0.06).

Discussion & Conclusion: The increase in NAA/Cr in the brainstem suggests that minocycline administration ameliorates in part the neuronal condition since NAA/Cr ratios have been previously reported to decrease by 20% in ALS patients (4). The increase in mI/Cr is indicative of a glial response to the medication. Therefore, these preliminary results are consistent with an improvement of the neuronal condition in the brainstem after a short minocycline treatment period.

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P107 SPATIAL VARIATION OF CEREBRAL METABOLITE ABNORMALITIES ALONG THE CORTICOSPINAL TRACT IN ALS DETERMINED USING MRSI

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Background: Proton magnetic resonance spectroscopic imaging (MRSI) can non-invasively detect neuronal loss or dysfunction by demonstrating a low signal from the neuronal marker N-acetylaspartate (NAA) relative to creatine (Cr) or choline (Cho). Previous studies have demonstrated decreased NAA/Cr in the motor cortex of ALS patients.

Objective: To evaluate the spatial variation of neuronal degeneration along the cerebral course of the corticospinal tract (CST) in ALS using MRSI.

Methods: Fourteen patients with probable or definite ALS as defined by the El Escorial criteria and 14 normal controls underwent MRSI on a 1.5T MR system using a 2D chemical shift imaging sequence. The MRSI slice was positioned in a coronal orientation parallel to the CST. Metabolite ratios were calculated along the rostral-caudal extent of the CST from the motor cortex to the cerebral peduncle, and for the entire CST.

Results: The mean bilateral NAA/Cho of the entire CST (4.95 ± 0.40 vs 5.75 ± 0.53, $p < 0.001$) was reduced more than NAA/Cr (1.90 ± 0.13 vs 2.09 ± 0.14, p=0.001) in ALS. The difference in these metabolite ratios was more prominent in the rostral portion of the CST; this was particularly impressive for NAA/Cho.

Discussion and conclusions: Cerebral metabolite ratios show a spatial variation normally along the CST.

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Spectroscopic indices of neuronal degeneration are more abnormal in the rostral portion of the CST. Although this would not be supportive of the dying-back hypothesis of neuronal degeneration in ALS, alternatively, it may be reflective of degeneration of neurons that do not contribute to the CST.

P108 DIFFUSION ANISOTROPY IMAGING IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with a progressive degeneration of upper and lower motor neurons. Diffusion tensor (DT) MRI imaging is a quantitative MRI technique. DT MRI allows the measurement of quantities reflecting the size, such as the apparent diffusion coefficient (ADC) and orientation such as fractional anisotropy (FA) of water-filled spaces in biological tissues.

Objectives: To determine water diffusion changes along pyramidal tracts in patients with amyotrophic lateral sclerosis and to assess whether DT MRI findings correlated with patients' disability.

Methods: Twelve patients with a diagnosis of ALS according to El Escorial criteria and nine age-matched controls underwent a diffusion tensor imaging examination. Disease severity was determined by means of the ALS Functional Rating Scale-Revised (ALSFRS-R). DTI-EPI data were acquired on a 3.0 Tesla GE Signa Infinity Scanner along 15 non-parallel directions.
The diffusion tensor Apparent Diffusion Coefficient (ADC) and Fractional Anisotropy (FA) (1), were computed from data and compared between groups on a voxel-by-voxel basis after normalization to MNI space. Each volume normalization and statistical comparisons have been carried out independently. All statistical analyses have been performed with $p < 0.01$. Correlation analyses of diffusion parameters and disease severity were performed using linear regression.

Results: Several brain areas showed significant changes on ADC and/or FA measures. Compared to controls, ALS patients had significantly lower FA ($p < 0.01$) in posterior limb of internal capsule, cerebral peduncle, corticospinal tract (CST) and corticobulbar tract. ADC values of ALS patients were significantly higher than those of controls in posterior limb of internal capsule, cerebral peduncle, CST and corticobulbar tract. Finally, a significant correlation was found between FA values and the ALSFRS-R. ADC values were inversely correlated with the disease severity.

Discussion and conclusions: Brain DT MRI in ALS patients allows detection of structural degeneration of the CST and corticobulbar tract. The correlation found between disability and ADC and FA values suggests that DT MRI may be useful adjunctive tool to monitor ALS evolution.

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P109 MEASUREMENT OF UPPER MOTOR NEURON DAMAGE IN MOTOR NEURON DISEASE BY TRANSCRANIAL MAGNETIC STIMULATION AND DIFFUSION TENSOR IMAGING

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Background: The measurement of upper motor neuron (UMN) involvement in motor neuron disease (MND) is difficult and no widely used objective technique is available. The sensitivity of neurological assessment of the UMN is confounded by lower motor neuron (LMN) signs.

Objective: Measurement of UMN involvement in ALS may help to monitor disease progression. Thus far, Transcranial Magnetic Stimulation (TMS), a method for neurophysiological assessment of upper motor neuron function and cortical abnormalities in MND and Diffusion Tensor Imaging (DTI) investigates corticospinal tract pathology in MND. We sought to develop a method of measuring UMN involvement using a combination of a novel threshold tracking TMS techniques combined with Diffusion Tensor Imaging.

Methods: A novel threshold tracking TMS protocol was implemented to measure cortical excitability using a BiStim magnetic stimulator. In the same sitting, axonal excitability was assessed by stimulating the median motor nerve at the wrist. Responses were recorded over the abductor pollicis brevis muscle.
All DTI studies were performed using a 3T Philips Intera with a 8 channel, phased array head coil and gradient coils (0–33mT/m). DTI was carried out in 15 directions. Tensor elements, eigenvalues, eigenvectors, diffusion anisotropy, diffusion constants and colour coded orientations were calculated using DtiStudio. Multiple regions of interest (ROIs) were selected within the corticospinal tract to enable corticospinal tracking. Studies were undertaken in 10 MND patients and 10 healthy controls. Measures of cortical excitability were correlated with the DTI parameters.

Results: There was significant reduction of short interval intracortical inhibition (SICI) in MND patients compared to controls (MND $-1.1 \pm 1.1\%$; controls, $7.1 \pm 1.2\%$, $P < 0.001$). Reduction of SICI was accompanied by

reduction in cortical silent period duration in MND patients (MND 0–202 ± 7.6 ms; controls 0–220.7 ± 10.2 ms, $P < 0.05$). Together, these findings confirm the presence of cortical hyperexcitability in MND.
The DTI studies have identified considerable differences between MND patients and controls in FA, MD and eigenvalues along the corticospinal tract between the internal capsule and midbrain in patients with MND, the main finding being within the left cerebral peduncle area (Ev1 0.00063 in MND and 0.00048 in control $p=0.0016$; Ev2 0.00045 in MND and 0.00033 in control, $p=0.0235$; FA 0.63 in MND 0.72 in control, $p=0.0235$ and trace 0.0026 in MND and 0.002 in control, $p=0.0025$). Preliminary findings suggest correlations between SICI, eigenvalues and FA. A novel algorithm was developed that is able to discriminate on the basis of TMS and DTI parameters between MND and control subjects.

Conclusion: The present study has confirmed the presence of cortical hyperexcitability in MND patients. Further, DTI studies established abnormalities in the corticospinal tract, consistent with possible axonal loss. There is a clear difference between the MND patients and control group in both the DTI and TMS studies.

P110 DIFFUSION TENSOR IMAGING IN PURE AND COMPLICATED HEREDITARY SPASTIC PARAPARESIS REVEALS REGIONAL ALTERATIONS IN THE CORPUS CALLOSUM

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Background: Hereditary spastic paraparesis (HSP) is a group of heterogeneous neurodegenerative disorders characterized by spasticity and weakness of the lower limbs in its pure forms (pHSP) while the complicated forms (cHSP) additionally present non-motor findings. Standard MRI of the brain shows a variety of abnormalities, and the most frequent finding is a thin corpus callosum (CC) in some cHSP forms. Diffusion tensor imaging (DTI) represents a promising experimental MRI technique for the analysis of white matter tract integrity. Based on the acquisition of echo-planar imaging sequences, the grade of tissue-specific anisotropy in each voxel can be estimated and visualized as a tensor. The main axis of the tensor therefore represents the orientation of fiber tracts, while the quantification of the orientational information is defined as fractional anisotropy (FA).

Methods: Twenty-one patients suffering from pHSP, 12 patients with cHSP, and 30 healthy age-matched controls underwent MRI including T1-weighted 3D and DTI sequences. By use of a novel DTI analysis software (tensor imaging and fiber tracking, TIFT), all individual patient data sets were normalized to a sequence- and scanner-specific DTI template. FA was calculated according to standard methods. Finally, TIFT provided quantitative whole brain-based statistical analyses by comparison of the FA-maps of each HSP-subgroup with controls and each other, respectively.

Results: There was a significant pattern at $p < 0.01$ of reduced FA in widespread areas of the medial and dorsal part of the CC in patients with cHSP compared to controls. The comparison of pHSP to controls revealed less extensive areas of reduced FA in the dorsal part of the CC. FA was also significantly reduced in cHSP compared to pHSP in ventral and dorsal CC regions. Surprisingly, no reduction of the FA of the pyramidal tract was observed at this thresholding.

Conclusions: Significant differences between patients with pure and complicated HSP compared to controls as provided by DTI led to new insights into the classification of HSP at group level by showing quantitatively assessable differences within the CC between the clinically defined subtypes.

P111 FUNCTIONAL CHANGES OF THE CORTICAL MOTOR SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS AND HEREDITARY SPASTIC PARAPARESIS

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Background: Both amyotrophic lateral sclerosis (ALS) and hereditary spastic paraparesis (HSP) are disorders significantly affecting corticospinal tract (CST). The pattern of additional impairment of other parts of the nervous system in these two conditions however differs. Functional imaging studies in patients with CST involvement have shown reorganization of motor circuitry.

Objectives: To investigate and compare functional changes of sensorimotor brain areas in ALS and HSP patients.

Methods: Nine ALS patients, 12 HSP patients and 13 healthy subjects were studied. Functional Magnetic Resonance Imaging (fMRI) was used to measure brain activation during right-hand finger tapping at 80% of each subject's maximum tapping rate. Image analysis was performed using general linear model and regions-of-interest (ROI) based approach. Weighted laterality indices were calculated for predefined ROIs to assess the lateralization of the motor system activation.

Results: ALS patients showed increased pre-supplementary motor area activation compared to healthy controls. Comparing ALS to HSP patients and HSP patients to controls, there were no statistically significant differences found using a voxel-wise comparison. Activation of the primary sensorimotor cortex was significantly less lateralized in both ALS and HSP patients compared to

controls. No differences in lateralization of activation were found between the two patient groups.

Conclusions: Our findings demonstrate less lateralized motor cortical activation in both ALS and HSP patients during motor tasks. Additionally, increased activation of the pre-supplementary motor area was found in the ALS group only. This may represent a specific pattern of reorganization of the cortical motor system, maybe due to abnormal excitability or loss of inhibitory control in the motor cortex in ALS, but not in HSP patients.

P112 MORPHOLOGICAL CHANGES OF THE LOWER CERVICAL SPINAL CORD UNDER NEUTRAL AND FULLY FLEXED POSITION BY MRI IN CHINESE PATIENTS WITH HIRAYAMA'S DISEASE

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Objectives: We examined the morphological changes of the spinal cord from the neutral neck to the fully flexed neck position in Chinese patients with Hirayama's disease (HD).

Methods: Routine examinations of magnetic resonance imaging (MRI) were performed on 25 HD patients and 31 health controls under neutral and fully flexed neck position. The mean anterior-posterior diameter (APD) and transverse diameter (TD) of the cervical cord at C6 level under different positions were measured and compared.

Results: Localized lower cervical cord atrophy and asymmetric cord flattening in a neutral neck position were highly suggestive of HD. The mean APDs were 5.6 ± 0.7 mm in HD patients and 6.7 ± 0.5 mm in controls ($p < 0.001$), and the mean TDs were 15.5 ± 1.2 mm in patients and 13.1 ± 0.7 mm in controls ($p < 0.001$). These changes seem to have certain relationship with longer disease duration, i.e. >18 months. Although displacement forward of the lower cervical spinal cord without significant compression was observed in young healthy people, the anterior shifting of posterior dura sac and lower cervical cord compression were distinctive and pathognomonic features in a fully flexed position of this disorder. The mean APDs were 4.3 ± 0.9 mm in HD patients and 6.0 ± 0.5 mm in controls on flexed position ($p < 0.001$).

Conclusions: There were dynamic morphological changes of the spinal cord during neutrality and full flexion. Straightforward at flexion MRI is the most characteristic finding of HD. Our results might be helpful for accurately diagnosing and assessing this disease.

P113 CEREBRAL PERFUSION ASSOCIATED WITH COGNITIVE FUNCTION IN PATIENTS WITH ALS: A LONGITUDINAL STUDY

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Background: ALS is a neurodegenerative disease affecting both the upper and lower motor neuron systems. Part of the clinical characterization of this disease now includes a cognitive aspect with varying degrees of severity. Although cognitive impairment (CI) is now a recognized manifestation of the disease, little is known about the temporal aspect of these changes, with even less of an understanding of the concomitant changes in cerebral perfusion.

Objective: To longitudinally assess concurrent changes in cognition and cerebral hemodynamics in patients with ALS as compared with control subjects.

Methods: The ALS group consisted of 13 patients (8 men, 5 women), age 34 to 63 years (mean, 50.6 ± 8.9 years). Eleven spousal controls (6 men, 5 women) were also recruited with ages ranging from 34 to 63 years (mean, 53.9 ± 8.2 years). Upon admission to the study, both groups completed a cognitive test battery and a CT Perfusion head scanning protocol that measured cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT). Patients were followed up with the same testing paradigm at two more time points over the next year, although some patients withdrew from the study due to medical or personal reasons. Time points for analyses were stratified based on the number of months post baseline measurements when the follow up studies were performed and were as follows: 4–6 months ($n_{ALS}=6$, $n_C=4$), 7–9 months ($n_{ALS}=3$, $n_C=2$), and 10–13 months ($n_{ALS}=5$, $n_C=4$). CI was defined by having two or more abnormal scores (>2 SD below the mean) on at least two different cognitive tests. Regional hemodynamic measurements were assessed by segmenting the CT images into the four lobes of the cortex. An ANOVA was performed to determine significant ($\alpha=0.05$) differences between the two groups for both cognitive tests and hemodynamic measurements at each time point.

Results: Cognitive testing, CBF and CBV indicated no significant difference between groups at any time point. MTT displayed an increasing trend in patients with ALS at all time points and was significantly higher ($p < 0.05$) in the ALS group at 10–13 months in the frontal, temporal and parietal regions.

Conclusions: Three of 13 ALS patients displayed mild CI, with the minimum requirement of two abnormal test scores, but declined no further throughout the duration of the study. Due to the mild nature of the impairment, groups were statistically similar with respect to cognition. The similarity in cognitive status between the two groups may account for the similar CBF and CBV measurements. However, MTT, the ratio of CBV to CBF, was found to be significantly different between groups. A trend for globally increased MTT was present at all time points in the ALS group when compared with the controls and became significant at 10–13 months. Increased MTT in the ALS group may be evidence for endothelial dysfunction not evident in discrete CBF and CBV measurements.

P114 STRATIFYING SUBGROUPS WITH DIFFERENT PROGRESSION RATE DETERMINED BY ELECTROPHYSIOLOGICAL TESTS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: We examined motor unit number estimates (MUNE) and compound muscle action potential (CMAP) amplitude as well as other quantitative tests in early stage of patients with amyotrophic lateral sclerosis (ALS). We tried to find an effective way to stratify the subgroups by different progression rate.

Methods: One hundred and twelve consecutive patients with definite or probable ALS were enrolled in the study. MUNE by semi-automatic incremental stimulation, CMAP amplitude, total Medical Research Council (MRC) manual muscle testing score, a revised ALS-functional rating score (ALSFRS-R), Appel ALS rating scale (AARS), and forced vital capacity (FVC) were performed at baseline and month 3, 6, and 12 in all patients.

Results: MUNE was correlated with CMAP amplitude ($r=0.72$, $P<0.01$) as well as MRC manual muscle testing score ($r=0.554$, $P<0.01$) in regionally concordant distal muscles. Both MUNE and CMAP amplitude correlated with ALSFRS-R ($P<0.05$) and AARS ($P<0.01$) significantly. A decrease in MUNE was observed at 3, 6, and 12 months compared with the baseline. The rate of change at 3 months was 50.47 %. CMAP amplitude, MRC manual muscle testing score, ALSFRS-R, and FVC declined sharply or smoothly. The decrease in MUNE over the first 3 months was significantly greater than other measurements. To evaluate further the utility of these tests, we arbitrarily divided the patients into three groups: (1) *rapidly progressive*: the rate of change of MUNE and CMAP amplitude during the first 3 months were more than 50% in 11 ALS patients; (2) *moderately progressive*: the rate of change of MUNE was greater than 50% while CMAP amplitude was less than 50%; (3) *slowly progressive*: the rate of change of MUNE and CMAP amplitude were both less than 50%. Comparing the rate of ALSFRS-R per year using one-way ANOVA showed a significant difference among the three groups ($F=37.39$, $P<0.01$), and between each of them by S-N-K method.

Conclusions: Our results suggested that MUNE and CMAP amplitude are helpful for stratifying patients with ALS based on their progression rate, which could enable the future results of clinical therapeutic trials more coincident for each subgroup.

P115 COMPARISON OF NEUROPHYSIOLOGICAL INDEX AND MUNE IN PATIENTS WITH ALS

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Background: Neurophysiological Index (NI) is derived from compound muscle action potentials, distal motor latency and F-wave frequency in the ulnar nerve / abductor digiti minimi (ADM). Recent studies have suggested that NI could be used as a sensitive measure of changes during the course of ALS. However, NI has some limitations and is not well-established. On the other hand, MUNE is an established method for functional measurement of ALS.

Objectives: This study aims to evaluate the correlation between NI and MUNE with functional status of ALS patients and to clarify which is more suitable measure as the surrogate marker for ALS.

Methods: The subjects of the study included 13 patients with ALS. NI and MUNE were recorded from the ADM by stimulating the ulnar nerve. The automated multiple point stimulation method was used for MUNE. Both MUNE and NI were tested twice by two experienced examiners, and averaged data were adopted as the results. Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS) and Norris scale were also scored simultaneously to assess the physical functions. In addition to the total scores of ALSFRS (ALSFRS-T) and Norris scale (Norris-T), we applied the sub-scores relating to the hand functions as ALSFRS-H and Norris-H. Both MUNE and NI data were compared with ALSFRS and Norris scale. Spearman's rank-order correlation coefficient was used for statistical analysis.

Results: MUNE was well correlated with ALSFRS-H ($\rho=0.750$), Norris-T ($\rho=0.768$) and Norris-H ($\rho=0.913$) significantly. The correlation of MUNE with ALSFRS-T ($\rho=0.481$) was not significant. On the other hand, NI was correlated significantly with Norris-H ($\rho=0.808$) only. The correlation of NI among ALSFRS-T ($\rho=0.202$), ALSFRS-H ($\rho=0.593$) and Norris-T ($\rho=0.583$) was not significant. In some cases at the early stage of ALS, MUNE showed some decline, although both ALSFRS and Norris score were nearly full marks.

Conclusion: MUNE was better correlated with the physical function scores than NI. Moreover, MUNE was more sensitive than those functional scales at the early stage, and we suggest MUNE might be a favourable surrogate marker for ALS.

P116 LONGITUDINAL ASSESSMENT OF RELIABILITY OF COMMONLY USED OUTCOME MEASURES IN ALS CLINICAL TRIALS

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Background: The Northeast ALS Clinical Trials consortium (NEALS) was founded in 1995 with 9 clinical ALS centers located in the Northeast USA. NEALS has expanded to include 65 sites in the USA and Canada, and has performed 7 multicenter trials since 1999. One of the missions of NEALS has been to establish standards for reliability of measurement for outcome measures employed in clinical trials, and to ensure that all sites meet these standards over time. To that end, reliability assessment has been required for all sites prior to initiation of any trial, and interim assessments have also been instituted. In this presentation, we summarize reliability results obtained for multiple outcome measures from 1999–2007.

Objectives: To demonstrate continuing proficiency in the performance of outcome measures used in NEALS clinical trials.

Methods: Reliability assessments were collected from 7 NEALS conducted multicenter clinical trials. Outcome measures whose reliability was assessed included quantitative motor testing using both the Tufts Quantitative Neuromuscular Evaluation and hand held dynamometry (QMT), Vital Capacity (VC), and Motor Unit Number Estimation (MUNE). For QMT and MUNE reliability assessments, normal control subjects were evaluated, while ALS patients were studied for VC reliability testing. Testing occurred prior to trial initiation, and at the midpoints of clinical trials.

Results: Each clinical trial involved the participation of different although overlapping groups of study sites. Reliability results are presented by site over time for sites participating in multiple trials. Reliability measures are also presented over time from trial to trial. Overall, average test retest variability for QMT was less than 10% for all trials using both HHD and TQNE. Average vital capacity variability was less that 10% for all sites at all evaluation times. MUNE has been employed in 2 trials; average variability for those trials was 11.5% and 11.7%.

Discussion: Over the course of 8 years, NEALS evaluators have sustained a high and consistent level of reliability for the major outcome measures used in clinical trials. New initiatives to improve reliability include a consortium wide program of yearly outcome measure validation, as well as continued efforts to improve reliability through education and formal training sessions.

P117 A NEW DEVICE TO MEASURE DISEASE PROGRESSION IN ALS CLINICAL TRIALS

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Background: Currently there are several potential therapies ready for ALS clinical trials. However, there are considerable resource constraints including cost, time, and especially human resource (patients and clinical researchers). An unmet challenge of the ALS research community is to conduct Phase II trials with improved time and cost efficiency to screen a large number of candidate drugs quickly.
Sample size is greatly influenced by the anticipated therapeutic effect, measurement variance, and the inter-subject variance. Use of sensitive, accurate outcome measures could greatly reduce variance while enabling us to detect modest treatment effects in a small sample.
Quantitative strength measures are sensitive indicators of disease progression in ALS. However, current methods are either too inconvenient or inadequate to accurately measure both strong and weak muscles. A new strength measurement device that easily and accurately measures modest changes in strength, could expedite future clinical trials by greatly reducing time, cost, and human resources.

Objective: To develop a convenient, easy-to-use device to accurately measure strength in subjects with ALS.

Methods: An analysis of a historical database containing isometric strength of 20 muscle groups from 421 patients with ALS confirmed that the following 12 muscle groups provided the most useful meaningful strength data: right and left elbow flexion, elbow extension, and grip strength; knee extension, knee flexion and dorsiflexion.
Engineers were contracted to design and build the first prototype designed to measure these muscle groups. Conceptual plans were developed based on the following criteria: 1) accurate and sensitive measures at all strength levels; 2) efficiency and ease of use and minimize missing data; 3) minimize test variability; 4) portable, 5) yields simple disease rates.

Results: We developed a new strength measurement system called: Accurate Test of Limb Isometric Strength (ATLIS). ATLIS consists of a folding platform with a post attached to the platform base. The platform can be raised or tilted using a pneumatic pump. A swivel chair with head

support can be secured to the platform. There are two mechanical arms with 3 degrees of freedom attached to the post. There is a stationary load cell at the distal end of the each testing arm that records the force produced by the muscle group being tested. Each load cell is surrounded by padded sleeve to assure proper stabilization during testing. Force is recorded and analyzed on a desktop or laptop computer, via a wireless connection.

Discussion and conclusions: Continued prototype development is underway to simplify the design and components of the ATLIS. Further clinical testing will be necessary to validate this instrument and to establish intra-rater and inter-rater test–retest reliability.

P118 THE ACCURACY OF ALSFRS SCORES CALCULATED FROM RETROSPECTIVE REVIEW OF CLINIC NOTES

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Background: Retrospective studies are a valuable research tool and can provide information about ALS more rapidly and efficiently than prospective studies. However, when comparing multiple groups retrospectively it is important to be able to account for disease severity and progression. The ALS Functional Rating Scale, Revised (ALSFRS-R) is a well established measure of functional impairment in patients with ALS and has been shown to be a strong predictor of survival in ALS.(1) It was designed to be completed during a face to face patient interview but can be accurately performed by telephone interview.(2) It is not known whether accurate ALSFRS-R scores can be generated from chart review.

Objectives: This study aims to compare ALSFRS scores obtained through patient interviews with ALSFRS scores generated from review of clinic notes to determine whether ALSFRS scores can be calculated retrospectively for use as a research tool.

Methods: This study was approved by the Institutional Review Board. Subjects with ALS were included for analysis if an ALSFRS score had been calculated by a trained nurse during a clinic visit and the patient also had a clinic note dictated by a neurologist within three months of the ALSFRS score. A research coordinator removed patient identifiers from clinic notes that corresponded to the time the ALSFRS score was completed in clinic. Four individuals independently used the clinic notes to generate ALSFRS scores. The four investigators comprised two nurses with extensive ALS experience, a physician, and a research technician. ALSFRS scores calculated from chart review were compared to the actual ALSFRS scores using summary statistics, Pearson Rank Correlation Coefficients, and Bland-Altman analysis.

Results: There were 103 subjects with prospectively obtained ALSFRS-R scores compared with retrospectively generated ALSFRS scores. The mean ALSFRS-R score from face to face interview was 41.9 ± 0.67 and the mean retrospective score was 42.8 ± 0.61. These were not significantly different. The actual and retrospective scores were significantly correlated with a correlation coefficient of 0.66. Agreement by Bland-Altman plot was generally good with only 4.8% of paired data falling outside of 2 S.D.of the difference.

Conclusions: ALSFRS scores generated from chart review appear to provide a reasonable estimate of scores obtained from patient interviews. While the retrospective ALSFRS scores are not a perfect substitute for actual ALSFRS scores obtained from patient interviews, this method may enable clinical researchers to account for functional impairment in retrospective studies.

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THEME 6 THERAPEUTIC STRATEGIES

P119 IS THE ALSFRS-R RATE OF DECLINE LINEAR OVER TIME?

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Background: The rate of decline of ALSFRS-R over time was used as a primary outcome measure in two recent large ALS clinical trials, in large part because analysis of data from previous studies indicated a high degree of linearity of the scale.

Objectives: To examine the linearity of the ALSFRS-R in the recent WALS Minocycline trial.

Methods: ALSFRS-R was measured monthly during a 4 month lead-in followed by a 9 month treatment phase where patients were randomized to 400mg/day of minocycline or placebo. Results were analyzed based on a linear mixed effects (lme) model where efficacy was measured by change-in-slope for treated compared to placebo during the treatment phase. Data from 205 placebo patients was used to investigate linearity using a bi-phasic linear model fit to each patient's data.

Results: The lme model estimated a pre-randomization slope of -0.81 units/mo for all patients during the lead-in. The post-randomization slope for the placebo group was -1.04 (28% steeper; $p=0.002$) and for the minocycline group was -1.3 (60% steeper; $p<0.001$). Individual patient analysis found 24% of placebo patients with significant ($p<0.05$) changes in slope following randomization. There were 35(17%) patients with significantly steeper slope and 14(7%) with significant flattening. Those with significant changes in slope did not differ from the remaining 156 placebo patients with respect to age, sex, weight, body mass index, symptom duration, site of onset (limb vs. bulbar), numbers of adverse events during the trial, use of riluzole, nor initial values of FVC, manual muscle tests (34 muscle groups) or ALSFRS-R. Analysis of ALSFRS-R subscores (bulbar, fine and gross motor, respiratory) at enrollment also did not differ between those with and those without significant changes in ALSFRS-R slope. Visual examination of individual patient plots suggested that the changes in slope developed gradually in most cases. These patterns suggested a logistic (backward S) model, however a bi-phasic linear model provided a better fit than the logistic model ($p<0.001$). These results are similar to those found in a reanalysis of data from the placebo arm (110 subjects) of the 10 month TCH346 trial where 18% of the placebo group showed a steeper rate of decline and 9% showed a flatter rate of decline, comparing the treatment phase to the lead-in.

Conclusions: About 25% of patients in the Minocycline and TCH placebo groups had non-linear rates of decline during the treatment vs lead-in phases of the trial. These findings underscore the need for a control arm when ALSFR-R is the primary endpoint.

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P120 ASSESSMENT OF THE CLINICAL SIGNIFICANCE IN THE CHANGE OF DECLINE IN ALSFRS-R

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Background: The ALSFRS-R (Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised) is an ordinal rating scale used to assess activities of daily living and respiratory function in people with ALS. It is easy to administer and commonly used in clinical trials of therapies for ALS. The ALSFRS-R declines monotonically with disease progression at a rate that is quite consistent across clinical trials. When used in clinical trials, a treatment related effect would be reflected as a change in rate of progression. However, there is no consensus regarding the amount of change that is deemed clinically significant.

Objectives: The primary objective was to survey ALS clinicians and researchers regarding what percentage reduction in rate of change of the ALSFRS-R score they would consider to be clinically meaningful.

Methods: A nine-question survey was provided to sixty-five ALS clinicians and researchers who are members of the Northeast ALS Consortium. They were asked to rate the clinical significance of 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% and 50% change in decline of the ALSFRS-R (slope vs. time) on a seven-point scale (1–7), where 1="Not at all clinically meaningful", 4="Somewhat clinically meaningful" and 7="Very clinically meaningful".

Results: Forty-two participants completed the survey. Of those, 90% rated a 20% change (4 or higher on the 7-point scale) in the ALSFRS-R score as the percentage in decline in which a moderate to substantially clinically significant

change is noted. All 42 participants (100%) endorsed a 25% or higher change (4 or higher on the 7-point scale) in the ALSFRS-R score. Ninety-three percent of the participants (39 members) maximally endorsed a 50% change in decline (7 on the 7-point scale) as very clinically significant. Thirty-one percent of the participants (13 members) endorsed a 10% change (4 or higher on the 7-point scale) as clinically significant, while 48% of the participants (20 members) endorsed a 15% change in the ALSFRS-R score as clinically significant.

Discussion and conclusions: This survey demonstrated that the majority of clinicians and clinical researchers surveyed believe that a therapy that resulted in a change of 20% or greater in the slope of ALSFRS-R would be clinically meaningful.

P121 WHAT ENDPOINT IS APPROPRIATE FOR ALS CLINICAL TRIALS?

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Background: Clinical trials for ALS typically use survival or rate of decline of function as a primary endpoint. Previous studies have shown that ALSFRS-R was predictive of survival but it is not clear whether one endpoint is more efficient than another when designing a trial.

Objectives: To use data from a recently completed clinical trial of minocycline to compare efficiencies of ALSFRS-R vs survival as endpoints.

Methods: During a 2 yr recruitment 470 patients were enrolled and 412 were randomized after 4 mos of lead-in to one of two study arms: 206 each to 400mg/day of minocycline and placebo for 9 months. Monthly testing included ALSFRS-R, FVC, and manual muscle tests (MMT) (34 muscle groups). Primary analysis was based on a linear mixed effects (lme) model where efficacy was measured by change-in-slope of ALSFRS-R for treated compared to placebo during the treatment phase. Secondary measures included rates of change in FVC, MMT as well as survival. We used standard methods for calculating sample size to compare efficiencies of endpoints based on changes in ALSFRS-R over time with that for survival.

Results: The lme model estimated a pre-randomization slope of -0.81 units/mo for ALSFRS-R in all 412 randomized patients. The post-randomization slope for the placebo group was -1.04 (a 28% increase; $p=0.002$) and for the minocycline group was -1.3 (a 60% increase; $p<0.001$). The difference in slope *change* is significant (-0.49 for minocycline vs. -0.23 for placebo; $p=0.005$) suggesting that this dose of minocycline led to greater decline in functionality compared to placebo. In contrast, a comparison of post-randomization survival showed no significant difference (median survival for minocycline 1.82 yr vs. 1.72 yr for placebo; $p=0.90$ log-rank test.). Similar, but less significant, results were obtained for FVC and MMT.

Thus, in this trial we detected a 22% difference in post-randomization slopes of the ALSFRS-R that did not correlate with a similar difference in survival. Could this difference be attributed to a lack of power for the survival endpoint? A standard power calculation formula with 470 patients recruited over 2 yr and followed 9 months after randomization, indicates 80% power to detect a hazard ratio of 0.66. This is equivalent to a 9 month difference in median survival, much larger than the riluzole effect of 2–3 months.

Conclusions: A phase III trial with ALSFRS-R as primary endpoint has excellent power to detect a therapeutic effect. If survival is the endpoint, the trial must be larger and/or longer to achieve the same power.

P122 APPLICATION OF A FINITE STATE MACHINE MODEL FOR MANAGEMENT OF MULTI-SITE CLINICAL TRIALS IN ALS

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Background: A Clinical Trial Management System (CTMS) for multi-site clinical trials in ALS automates many aspects of project management, and in doing so provides standardization of reports, improves communication, and provides transparency for audits. A finite state machine (FSM) is a model of behavior composed of a finite number of states, transitions between those states, and actions. Integrating the FSM approach to the CTMS, allows customization according to study design specifications.

Objective: To apply the FSM concept to the CTMS for customizable workflows in protocol deviation reporting, protocol exception requests, serious adverse events reporting, and electronic case report form change requests.

Methods: The FSM workflow describes the movement of a document through different states of its lifecycle. A user role determines the actions that dictate the succeeding directions of the document. At each point of this flow the document can be described by a specific state. Variations in coordination center configuration, as well as study design, have required different workflows for the same aspects of management. Causes of variations included: 1) Degree of sponsor participation, 2) Number of principal investigators and levels of their involvement, 3)Medical monitoring structure including medical specialists' involvement.

Results: We have applied CTMS with the FSM model for three multi-site clinical trials in ALS. Specific variations that have been allowed include:

Serious Adverse Events: i) Safety Monitor in addition to Medical Monitor, ii) Medical Specialist Monitor who is notified and responds only to specific adverse events
Protocol Exception Request: Allow for Data Safety Monitoring Board members to act as reviewers to provide their feedback to the Project Manager
eCRF Change Request:i) Review by data management before submission to IRB, ii) Approval required by the Project Manager rather than the Principal Investigator

Conclusion: Integrating the FSM principles into the original CTMS development proved to be the correct choice as it allows flexibility in configurations of the documents workflows. Customization could be expanded not only to future studies but also to new documents and reports.

P123 COMPARISON OF CONTROLLED MEDICAL VOCABULARIES FOR ADVERSE EVENT RECODING IN A MULTI-CENTER CLINICAL TRIAL IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Accurate adverse event (AE) reporting is essential for successful conduct of clinical trials. The Neurology Clinical Trials Unit at the Massachusetts General Hospital historically reported AEs for its clinical trials in ALS using the FDA's Coding Symbols Thesaurus of Adverse Reaction Terms (COSTART). Recently the National Institutes of Health advanced another controlled medical vocabulary, Common Terminology Criteria for Adverse Event (CTCAE).

Objective: To compare COSTART and CTCAE systems in recoding of AE's using AE data from the published clinical trial of celecoxib in ALS.

Methods: The COSTART dictionary is based on a glossary of AE terms. Corresponding AE's are first matched to terms in the COSTART glossary and then COSTART automatically assigns each AE to multiple pathological or anatomical organizational levels or groups. The CTCAE dictionary uses four organizational levels and its primary organization is based on pathophysiological and anatomical categories to facilitate location of related AE's. The coder maps the term to a relevant organizational level or group and selects from a list of available terms and assigns a grade of severity to each AE.
A total of 1349 AE's were observed during the clinical trial of celecoxib in ALS (27 sites, 300 subjects) and coded in COSTART terms. Using CTCAE, we recoded 60% (791/1349) randomly selected AE's from the trial. Blinding was maintained throughout the process. Organizational levels of all AE's recoded in both COSTART and CTCAE were then compared.

Results: After coding 791 AE's using both COSTART and CTCAE and then mapping them to their corresponding category, 59% (470/791) of AE's were found to be organized into a different pathological or anatomical grouping. Among the 474 AE's: 216 (47%) were grouped by COSTART into PATHOLOGICAL whereas CTCAE grouped them into ALLERGY/IMMUNOLOGY, PAIN, INFECTION and DERMATOLOGY/SKIN; 170 (21%) were grouped by COSTART into NONSPECIFIC while CTCAE grouped these AE's into GASTROINTESTINAL, PULMONARY, CONSTITUTIONAL SYMPTOMS and PAIN; 46 (6%) were grouped by COSTART into AUTONOMIC NERVOUS SYSTEM while CTCAE grouped these into GASTROINTESTINAL; 21 (4%) were grouped by COSTART into CARDIOVASCULAR while CTCAE grouped them into LYMPHATICS. 19 (4%) were grouped by COSTART into NERVOUS SYSTEM while they were grouped by CTCAE into PAIN.

Conclusion: Our data demonstrates a wide difference in how COSTART and CTCAE organize and report AE data. We found that the CTCAE grouping of terms is more accurate and provides more options for the coder to assign relevant groups and grades to each AE. Online utilization of CTCAE by the site users also eliminates interpretation error of COSTART approach.

P124 DESIGN AND IMPLEMENTATION OF A MULTI-PHASE, ADAPTIVE-DESIGN CLINICAL TRIAL FOR SUBJECTS WITH ALS

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Background: Historically, adaptive or alternative-design clinical trials have been utilized in oncology and HIV research as an efficient method of drug discovery. These adaptive-design trials have not traditionally been used in clinical trials for ALS. The NIH-funded *Clinical Trial of Ceftriaxone in Subjects with ALS,* combines Phase I-II-III clinical trials into one multi-phase trial, including interim analyses and dosage adjustments based on these analyses.

Objectives: To safely speed the drug discovery process by combining pharmacokinetics, safety, tolerability, and efficacy into one single study with a novel, adaptive design.

Methods: We employed a multi-phase, adaptive design to more efficiently test ceftriaxone in subjects with ALS. This design allows the same subjects to participate in all phases of the study. Stages 1 and 2 of the study, which are currently in progress, will enroll 60 subjects to evaluate pharmacokinetics (stage 1), safety, and tolerability (stage 2) over 20 weeks of treatment. Stage 3 of the study will include the original 60 subjects and enroll an additional 540 subjects (for a total of 600 subjects) to evaluate

efficacy, with a primary outcome of survival, while continuing to evaluate long-term safety and tolerability. This type of study design requires an experienced coordination and data management team and validated, user friendly electronic data capture and clinical trial management systems.

Results: The FDA required that the preclinical package support a phase III efficacy study. This included chronic animal toxicology data. To meet this requirement, we conducted two IND supportive rodent studies. The first, a short-term toxicity and dose-finding study, identified the maximum tolerated dosage for chronic subcutaneous administration in rodents (doses up to 1.0 g/kg/day tested). Subsequently, a 6-month rodent toxicology study was conducted, beginning in the summer of 2006. The FDA allowed the human study to proceed concurrent with the 6-month rodent study. Enrollment for stage 1 and 2 of the human study began in late August of 2006 at 9 US sites. Data is entered into an electronic data capture (EDC) system (Pharmacontent, Inc) and the trial is managed using a clinical trial management system (CTMS) we developed.

Discussion: Using the experience from other clinical research specialities, ALS researchers have begun to utilize these ''alternatives'' to traditional phased clinical trials. The use of adaptive-design clinical trials may speed the drug discovery process. *The Clinical Trial of Ceftriaxone in Subjects with ALS* is one such study, utilizing a multi-phase, adaptive design to combine multiple aspects of the clinical trials process. Although it requires a significantly increased amount of supportive preclinical toxicology data, we expect that this type of design will be a useful tool to increase the efficiency of clinical trials in ALS research.

P125 – ABSTRACT WITHDRAWN

P126 THE FIRST TRIAL OF AAV GENE THERAPY IN AMYTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. The deficiency of neurotrophic factors is proposed in motor neuron death in ALS. Angiogenic factors (vascular endothelial growth factor (VEGF), angiogenin) have been implicated in the pathogenesis of ALS. Genetic studies revealed the specific haplotypes of VEGF and angiogenin genes in ALS patients. The decrease of VEGF levels in biological fluids were detected in ALS patients. New animal models of motor neuron disease with mutations in the VEGF gene have been created.

Objectives: The aim of our study was to estimate the clinical efficiency and safety of adenoassociated virus (AAV), that encodes VEGF and angiogenin, in ALS patients.

Materials and methods: Ten patients with sporadic ALS were included in our study. Five patients (four males and one female) with mean age 41.8 ± 4.1 years, mean duration of disease 17.6 ± 8.8 months with cervical onset of ALS received intramuscular injections of AAV, encoding VEGF and angiogenin, monthly in six muscles (m. trapezius, m. deltoideus, m. quadriceps) during one year. The control group included five patients (four males and one female) with cervical onset, with mean age 52.0 ± 10.8 years, mean duration of disease 15.0 ± 11.7 months. These parameters did not differ from the treatment group. The control group received monthly intramuscular injections of buffer solution in six muscles. The endpoints of the trial were forced vital capacity (FVC), ALSFRS score and dynamics of weight in ALS patients.

Results: In both groups an increase of neurological disturbances and a decrease of FVC were revealed during the first year of the trial. The rate of progression of disease didn't significantly differ between the two groups. In the control group two patients died 10 months after the beginning of the study, while all patients in the treatment group, who received AAV gene therapy, are alive. No serious adverse effects were revealed. The patients that received the gene therapy, experienced flu like symptoms for several hours after the injection. Our study is being continued.

P127 A MULTICENTRE TRIAL ON G-CSF IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Granulocyte-colony stimulating factor (G-CSF) is widely used for stimulating the mobilization of

bone marrow-derived cells (BMCs) into blood. These cells have been demonstrated to have protective effects on motor neurons in animal models of ALS, possibly related to the endogenous synthesis of neurotrophic factors. They have also shown the ability to transdifferentiate into neurons and glia both *in vitro* and *in vivo*.

Aims: To evaluate the safety and tolerability, and haematological and cerebrospinal fluid (CSF) response of the treatment with G-CSF in ALS patients.

Methods: Patients with definite, probable or probable laboratory-supported ALS were included. Inclusion criteria were: age between 40 and 65, disease duration under 12 months, moderate disability, and FVC% $\geqslant$ 80% of expected. After a run-in period of 4 months, patients underwent 4 cycles of treatment with G-CSF. G-CSF was administered subcutaneously at the dose of 5 μg/kg, twice a day, for 4 consecutive days. From the third day of G-CSF treatment, 22.5 g of mannitol 18% solution, four times a day for 5 days, was given intravenously in order to permeabilize brain-blood barrier. A lumbar tap was performed at the recruitment (time -4 months), and again at the end of the first and third treatment. At time -4 a baseline haematological evaluation was performed, with blood cell count and bone marrow aspiration. The evaluation of mobilization of BMCs was performed assessing the number of circulating CD34+ cells, at each cycle, at day 0, during the four days of G-CSF administration, and for at least 2 days after the drug intervention. Evaluation of treatment safety and tolerability was performed at each cycle. Pre-treatment (from time -4 to time 0) and treatment (from time 0 to time 12) progression rates of disability (MRC, ALS-FRS-R) and QoL scores (McGill QoL Questionnaire) were compared.

Results: Twenty patients have been recruited (12 men, 8 women; mean age 54.2 years) and have been evaluated after 2 cycles of G-CSF. The mean baseline level of CD34+ in the blood was 3.15/μl. During the treatment period all patients showed an increase of CD34+ up to a mean of 55.2/μl, with a peak on the fourth day. The haematological response was substantially similar at each cycle of treatment. The main side effects were: an increase of growth hormone level in one patient, and a deep vein thrombosis in one leg in one patient. In both cases the study treatment was continued. Most patients reported nausea and flu-like symptoms during the drug administration, but these were mild and promptly controlled by acetaminophen.

Conclusions: The treatment with G-CSF in ALS patients is safe and well tolerated, with few and reversible adverse effects. The final evaluation of the data will be performed in November 2007.

P128 INVESTIGATION OF THE THERAPEUTIC EFFECTS OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: A PILOT STUDY

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Objectives: The present study was to investigate the safety and efficacy of G-CSF treatment in ALS patients (a pilot study).

Methods: Thirteen ALS patients were enrolled after they had given their informed consent to participate in the study. The inclusion criterion was a diagnosis of SALS with duration less than 3.5 years. The exclusion criteria included tracheotomy, artificial respiration and dyspnea. RhG-CSF injections were administered setting of 2μg/kg/day once per day via subcutaneous for 5 days. The patients were observed for 9 months (from 3 months before administration to 6 months after administration) at least. The primary outcome measure was the change of ALSFRS-R during three periods – 3 months prior to the treatment, 3 months after treatment, and 3 to 6 months after treatment. The secondary outcome measure was average CMAP amplitude.

Results: Three of the patients died in the 6 months after the first treatment. The mean age was 51.69 ± 16.28 years. Disease duration was 18.38 ± 12.46 months, including initial symptoms of bulbar in 3 cases and extremity in 10 cases. Initially their ALSFRS-R was 27.31 ± 9.446, AARS was 76.15 ± 30.54, average CMAP amplitude was 3.09 ± 2.01. During the 3 months after the treatment period, the decline of the ALSFRS-R was significantly better than that in the 3 months prior to G-CSF administration (-0.6154 ± 1.1209 vs -3.2308 ± 2.8330, $P=0.012$), the change of the ALSFRS-R in 3 to 6 months after treatment was significantly worse than that in the 3 months after G-CSF administration (-1.6 ± 1.0750 vs -0.6154 ± 1.1209, $P=0.009$) too. Secondary analysis by repeated measures analysis of variance concludes similarly, test of within-subjects effect gives a P value of 0.023. Thus, treatment with G-CSF appeared to reduce the rate of decline of ALSFRS-R. The level of CMAP is an objective index avoiding placebo effect. Wilcoxon signed rank test indicates the decline of CMAP amplitude during 3 months after rhG-CSF administration was significantly better than that in natural course in 3 months prior to administration (-0.6914 ± 1.1762 vs 0.0591 ± 0.6041, $P=0.023$), the change of the CMAP amplitude in 3 to 6 months after treatment was worse than that in the 3 months after administration (-0.3426 ± 1.4997 vs 0.0591 ± 0.6041, $P=0.093$), but not significant.

Conclusions: We found that the decrease of the ALSFRS-R during the 0–3 months after treatment period was significantly better than that in the -3–0 months prior to the start of treatment. This result suggests that G-CSF may delay the progression of functional disturbances in

ALS patients. The decline of ALSFRS-R in 3–6 months was significantly worse than that in 0–3 months, but better than that in −3–0 months of natural course. This phenomenon indicated the possibility of a residual effect in 3–6 months after treatment. Our trial showed the change of average CMAP amplitude during 0–3 month after treatment period was significantly better than that in the −3–0 month prior to the start of treatment, the mean value even become positive. The decline of average CMAP amplitude in 3–6 months after treatment was negative and worse than that in 0–3 months, but better than that in −3–0 months. It is necessary to confirm the efficacy of rhG-CSF in a randomized, placebo-controlled, double-blinded design. We have registered (NCT00397423), and are conducting the trial at present.

P129 EVALUATION OF TREATMENT WITH RASAGILINE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Rasagiline is a new anti-parkinsonian agent with neuroprotective properties *in vitro* and *in vivo*, which increased survival in transgenic mice with SOD1 mutations. It has minimal side effects and is well tolerated.

Objectives: To analyze retrospectively the efficacy and safety of rasagiline in ALS.

Methods: Eighteen patients (13 males) aged 56 ± 14.3 years, with clinically definite or probable amyotrophic lateral sclerosis (ALS) were treated off-label with rasagiline 2 mg as add-on to riluzole 100 mg daily. Patients were evaluated every 3 months using the ALSFRS-R scale and manual muscle testing (MMT). For 9 patients clinical evaluations were available for 9-14 months before the start of rasagiline study. The rate of deterioration of ALSFRS-R scores in rasagiline-treated patients was compared to that of 25 randomly chosen ALS patients, treated with riluzole only during the same time period in our clinic. Baseline ALSFRS-R was slightly lower in the rasagiline group (33 ± 10.7) than in the riluzole-only group (40 ± 5.4).

Results: Mean time on rasagiline was 9.5 ± 5.8 months (range 2.23–24.94). The mean slope of ALSFRS-R deterioration for rasagiline-treated patients was 0.68 ± 0.37/month, compared to a slope of -1.02 ± 0.60 in the riluzole-only group, and a slope of ~ -1 reported in the literature. In patients with pre-treatment data, the mean deterioration slope was -1.11 ± 0.27/month before rasagiline onset and 0.62 ± 0.27 thereafter. For 12 patients treated >6 months with rasagiline, the deterioration slope was -0.82 ± 0.97/month during the first 6 months, and -0.56 ± 0.51 thereafter, suggesting an accumulated effect of rasagiline. MMT scores did not reveal positive effects of rasagiline, possibly due to the non-linearity of this test. No significant side effects of rasagiline were reported.

Conclusion: Despite limitations of this small, non-blinded, retrospective analysis, it appears that rasagiline has positive effects on the rate of ALS deterioration. Further investigation of this drug as a potential treatment for ALS is warranted.

P130 A RANDOMIZED, PLACEBO-CONTROLLED, SEQUENTIAL TRIAL OF VALPROIC ACID IN ALS

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Background: Valproic Acid (VPA) is a FDA-approved drug with well-known pharmacokinetic and toxicity profiles. VPA exerts is action targeting multiple neuronal mechanisms, which may also play a role in ALS pathogenesis. Spinal muscular atrophy (SMA) is caused by a homozygous deletion of the survival motor neuron (SMN) 1 gene. SMN2, the nearly identical copy of SMN1, plays a disease modifying role in SMA. Previous studies investigated the possible role of SMN in ALS. A pooled analysis showed that low copy numbers of SMN1 and SMN2 are significantly associated with the risk of ALS. It was concluded that SMN genotypes producing less SMN protein increase susceptibility and severity of ALS. Increasing expression of SMN protein may alter ALS disease course and extend survival. A number of in vitro studies suggest that valproic acid (VPA) may increase SMN mRNA and protein expression by acting as histone deacetylase inhibitor.

Objectives: We performed a randomized placebo-controlled, sequential clinical trial to investigate the efficacy of VPA on survival in ALS and to study the effect of VPA on SMN mRNA and protein expression.

Methods: Between April 2005 and January 2007, 163 patients with probable, probable-laboratory supported or definite ALS were randomly assigned to receive either VPA or placebo 1500 mg daily. A sequential trial design was used with death, persistent assisted ventilation, or tracheostomy as primary end points. Secondary outcome measurement was rate of decline of functional status. All patients were genotyped for SMN1 and 2 copy number. Before starting trial medication and after four months, blood samples were drawn from all patients. mRNA and protein were extracted from peripheral blood mononuclear cells. SMN mRNA expression was measured using real time PCR and SMN protein was quantified using an SMN specific sandwich ELISA.

Results: VPA did not affect survival (cumulative survival probability of 0.72 in the VPA group vs 0.88 in the placebo group at 12 months, and 0.59 in the VPA group vs 0.68 in the placebo group at 16 months), or the rate of decline of functional status. VPA intake did not cause important adverse reactions. Treatment with VPA did not change SMN mRNA and protein expression.

Discussion and conclusions: This placebo-controlled trial showed no evidence for a beneficial effect of VPA on survival or disease progression in patients with ALS. We did not find evidence for an effect of VPA on SMN mRNA and protein expression.

P131 THE LONG-TERM EFFECT OF EDARAVONE IN ALS PATIENTS

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Background: Edaravone has been reported to be beneficial for ALS treatment in a clinical trial of 6 months repeated administration (1).This time, we examined ALS patients' background and bodily functions and we investigated effect of the long-term edaravone treatment in the Yoshino neurology clinic.

Objectives: Forty-nine ALS patients were recruited from the clinic (male: 30, female: 19, average age 61.1 ± 11.13 years old, and upper extremity palsy (UEP) 22 subjects, lower extremity palsy (LEP) 14 subjects, and bulbar palsy (BP) 13 subjects.

Methods: We recorded the age, duration of the disease, ALSFRS-R, %FVC, walking ability, use of NIPPV or not, and the presence of a tracheotomy. Moreover, we made a comparative study among 22 subjects (male: 10, female: 12 and UEP 8, LEP 7, BP 7) who survived three years or more by administrating the edaravone or not.

Results: The results show that the mean age of the onset of the disease was 58.58 ± 11.55 years old, and the duration of the disease was 3.46 ± 2.41 years. ALSFRS-R was 32.57 ± 9.53 points, %FVC was 74.6 ± 33.5, 26 subjects were able to walk independently, 15 subjects used NIPPV and 2 subjects had tracheotomy in this clinic. Among 22 subjects who survived 3 years or more, 9 subjects (4 UEP, 1 LEP, and 4 BP) received edaravone and in the non-edaravone group 13 subjects did not (4 UEP, 6LEP, and 3 BP). Morbidity period from onset was 4.4 ± 1.2 in the edaravone group and 5.9 ± 2.5 years in the non-edaravone group. ALSFRS-R was 31.3 ± 6.7 in the edaravone group and 25.3 ± 9.1 in the non-edaravone group. %FVC was in the edaravone group 79.2 and 56.6 in the non-edaravone group. The average period of receiving edaravone was 1.85 ± 1.87 year.

Discussion: Although this is not a randomized study, edaravone might inhibit the progression of the disease in patients who survived three years or more. We will also report the effect of rehabilitation for ALS patients who received edaravone.

Reference:

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P132 SHOULD PATIENTS WITH ALS CONTINUE TAKING STATIN MEDICATIONS?

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Background: Statin medications (HMG-CoA Reductase Inhibitors) for elevated cholesterol are one of the most commonly prescribed medications worldwide. A significant proportion of patients with ALS are taking statin medications for high-cholesterol, but the effect on disease progression is unknown. Myotoxicity is a well documented side-effect of statin medications with symptoms ranging from myalgias and a mild elevation in serum creatine kinase (CK) to rhabdomyolysis. The myotoxic side-effects of Statin medications occur more frequently in patients with metabolic muscle diseases (1), but it is unknown if this increased risk would also apply to patients with motor neuron disease. Alternately, cholesterol mediated oxidative stress-induced motor neuron death has been proposed as a pathogenic mechanism in ALS and statins may be neuroprotective (2). Presently, there is little data to guide patients with ALS on whether or not they should continue taking statins.

Objectives: The aim of this study was to determine if statin medications affect the rate of disease progression, frequency and severity of muscle cramping, and serum CK levels in patients with ALS.

Methods: We conducted an observational cohort study with statin medication as the predetermined exposure variable and the rate of decline of the revised ALS functional rating scale (ALSFRS-R) as the primary outcome measure. Patients diagnosed with clinically probable or definite ALS were assessed at 3 month time intervals recording the ALSFRS-R and muscle cramps scores.

Results: The study included 131 patients with clinically probable or definite ALS followed from January 2006 to June 2007. Thirty-two patients (24%) were taking statin medications and 99 (76%) were in the control group. After adjusting for covariates, we found a highly significant increase in the rate of decline in the ALSFRS-R for the statin group (1.29 units/month) compared to the control group (0.77 units/month; $p=0.0015$). Patients in the statin group also reported significantly greater muscle cramp frequency ($p<0.0001$) and severity ($p=0.0005$). CK values were lower in the statin group, but no significant difference was found.

Conclusions: This observational study demonstrates that statin medications are associated with an increased rate of

disease progression in patients with ALS. Statins also appear to significantly increase muscle cramp frequency and severity. These findings indicate that statin medications may be harmful in patients with ALS and clinicians should consider discontinuing or replacing these medications in the context of ALS disease progression and cardiovascular risk.

References:
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2. Cutler R, Pedersen W, Camandola S et al. Ann Neurol. 2002; 52: 448–457.

P133 THALIDOMIDE CAUSES SINUS BRADYCARDIA IN ALS

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Objective: Neuroinflammation contributes to motor neuron degeneration in ALS. Thalidomide (THL) shows potent anti-inflammatory properties and increased the life span in ALS transgenic mice. Therefore, thalidomide was suggested as therapeutic intervention for the treatment of ALS. We conducted a pilot, randomized trial of THL in patients with ALS to assess safety, feasibility, and preliminary estimates of treatment efficacy.

Methods: Patients were randomized to THL in combination with riluzole (n=18) or riluzole alone (n=19). THL was initiated at 100 mg for 6 weeks. Thereafter, the dose was increased every week by 50 mg until reaching the dose of 400 mg/day and continued for 12 weeks.

Results: Within 12 weeks, 9 THL patients (50%) developed bradycardia defined as a heart rate below 60 beats per minute (bpm) and ranged from 46 to 59 bpm. Mean heart rate dropped by 17 bpm with THL treatment. Severe symptomatic bradycardia of 30 bpm occurred in one patient. A further patient died from sudden unexpected death. The study was terminated prematurely for safety concerns. The secondary outcome variables showed similar results for both groups.

Conclusion: Bradycardia was the most common adverse event of THL treatment in ALS. THL-related bradycardia does not appear to be ALS-specific. However, it is well conceivable that the unexpected frequency and severity of THL-induced bradycardia may be related to the subclinical involvement of the autonomic nervous system in ALS. The cardiac toxicity discourages further trials and compassionate use of THL in ALS. ClinicalTrials.gov Identifier: NCT00231140

P134 COMBINED ANALYSIS OF CREATINE MONOHYDRATE TRIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Preclinical data suggests that creatine is neuroprotective and prolongs survival in animal models of ALS. Clinical benefit at 5 and 10 grams has not been demonstrated in human ALS trials, however, survival analysis across comparable trials has not been performed and may shed new light on the assessment of creatine in ALS.

Objective: The authors sought to examine the effect of creatine on survival in patients with ALS.

Methods: Trials were identified by PubMed search and enquiry from authors of trials and Avicena (manufacturer). Selection criteria included randomized, placebo-controlled trials in adult subjects with ALS that employed creatine. For eligible trials, based on methodological quality and comparability of study population, data was collected from investigators and analyzed using a random effects model. An estimate of treatment effect on survival was assessed for each trial and on pooled data using a Cox Proportional Hazards model, controlling for trial effect.

Results: Three trials were identified and included a total of 386 patients, 191 creatine-treated, 195 placebo-treated patients. The first trial (NEALS) compared 5 gm creatine per day to placebo over 6 months in 104 patients (54 creatine-treated, 50 placebo-treated). A second trial (CMC) compared 5 gm creatine per day to placebo for 9 months in 107 patients (53 creatine-treated, 54 placebo-treated). The third trial (Netherlands) studied the effect of 10 gm creatine per day versus placebo in 175 patients with ALS (88 creatine-treated, 87 placebo-treated). Mean baseline ALSFRS differed significantly between trials ($p<0.0001$) (NEALS: 41.8 ± 5.6; CMC: 36.1 ± 6.1; Netherlands: 30.5 ± 5.1), as did screening predicted FVC/VC ($p<0.0001$) (NEALS: 84.2 ± 19.9; CMC: 77.3 ± 20.9; Netherlands: 89.9 ± 18.5). In addition, overall survival was significantly worse in the Netherlands trial compared to NEALS and CMC ($p=0.02$, HR=2.6, 95% CI 1.1–5.9). Creatine showed a trend towards increased survival in the pooled data, but the treatment effect did not reach significance ($p=0.10$). The pooled hazard ratio was 0.679 (95% CI 0.426–1.080) for the creatine group relative to the placebo group. In other words, there was a 32% reduction in the hazard ratio for patients taking creatine. Median survival for the creatine group showed a 1.47-fold improvement over placebo.

Conclusions and relevance: A pooled analysis of three randomized, placebo-controlled studies suggests a trend

towards increased survival in patients taking creatine although no benefit on functional measures of disease progression was detected in any trial and the treatment effect did not reach significance. The certainty of these results are further limited by sources of error that could be minimized in a single, large, well-designed clinical trial of creatine in patients with ALS.

P135 A PILOT TRIAL OF LEVETIRACETAM FOR CRAMPS, SPASTICITY AND NEUROPROTECTION IN PATIENTS WITH MOTOR NEURON DISEASES

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Background: Levetiracetam (Keppra) is used to treat partial onset seizures. Its biological effects suggest it might be useful in treating 3 aspects of human motor neuron diseases (MNDs) for which no effective therapy exists: cramps, spasticity and disease progression.

Objectives: 1. To determine the stability of cramp and spasticity scores during a 3 month pre-treatment baseline period in patients with MNDs. 2. To assess the safety and tolerability of Levetiracetam over 9 months of treatment in patients with MNDs. 3. To determine whether treatment with Levetiracetam is associated with a reduction in cramps, spasticity or disease progression.

Methods: Open-label, phase 2 trial of 20 adult patients with MND (ALS, PLS or PMA) at Duke University ALS Clinic. Eligible patients have cramps with average severity 50/100 points, are able to provide informed consent, have normal renal function and are on a stable riluzole dose. Exclusions include pregnancy, unstable medical illness, dementia, drug abuse or non-compliance. The first 3 months of the study are a baseline period. Over the remaining 9 months patients take Levetiracetam at increasing doses up to 3000mg per day. Outcome measures include adverse events, tolerability, cramp-pain-severity score, cramp-frequency score, modified Ashworth Spasticity Score, Penn Spasm Score, FVC, ALSFRS-R and MMT.

Results: Enrollment began May 2006 and 21 patients have now passed screening. Prior to starting the trial, one patient recalled prior treatment with Levetiracetam and was thus disqualified. The last patient enrolled in November 2006. In terms of demographics, there are 10 males and 10 females. Ages range from 38 to 78. There is 1 non-white subject. ALS, PLS and PMA are all represented. Thus far Levetiracetam appears safe and well tolerated. There have been 4 serious adverse events: appendicitis, PEG tube infection, severe dehydration due to diarrhea and urinary tract infection, and death. None of these were considered related to drug. Non-serious adverse events are consistent with those seen in prior studies of Levetiracetam. In most patients, cramp scores have been stable during the baseline period. In some, there is a decrease in cramp scores coincident with starting Levetiracetam.

Discussion: The results of this study will impact the design of future studies using similar outcome measures. If Levetiracetam continues to appear safe and tolerable, and is associated with an improvement in any study outcome measures, a double-blind, placebo-controlled crossover trial will start in 2008.

P136 PYRIMETHAMINE AS A THERAPY FOR SOD1 ASSOCIATED FALS: EARLY FINDINGS

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Background: Three percent of ALS patients have a familial form of the disease (FALS), caused by a mutation in the gene coding for the free radical scavenging enzyme copper/zinc superoxide dismutase (SOD1) and phenotypically identical to the sporadic illness. Inhibiting expression of the SOD1 gene prevents transgenic ALS animals from developing the disease. Increasing or decreasing the number of mutated genes proportionately speeds or slows the progression of the disease. Therefore, reducing SOD1 levels in patients with SOD1 associated FALS may be a promising therapeutic approach. Through an extensive in vitro screening program for medications having the ability to reduce SOD1 levels, several molecules that reduce SOD1 protein levels are known. One of the most potent molecules is pyrimethamine, an FDA approved medication used for the treatment of malaria and toxoplasmosis. Pyrimethamine dramatically reduces SOD1 levels in laboratory tests.

Objectives: To describe changes in SOD1 levels and muscle strength in FALS patients receiving pyrimethamine over an 18 week time period.

Methods: A 61 year old man developed progressive leg weakness and fatigue manifested as difficulty climbing stairs, rising from chairs and frequent tripping. There was no hand weakness. His mother's nephew (sister's son)-**cousin**, age 72, has ALS and currently is on a ventilator with little limb movement. Examination showed weakness of hip flexion and wrist extension. Reflexes were overactive in the arms and legs with bilateral Hoffmann signs and one beat of ankle clonus bilaterally. EMG showed spontaneous activity in multiple muscles in the arms and legs in the form of positive sharp waves and fibrillation potentials. Recruitment pattern was reduced in all muscles tested. Laboratory studies revealed an SOD1 mutation D90A on one allele; the other allele was normal. The D90A mutation in the heterozygous form is associated with both progressive motor neuron disease and non progressive multifocal disease. Progressive weakness with overactive reflexes in association with the D90A mutation established

the diagnosis of SOD1 associated FALS. Pyrimethamine was started at 25 mg per day and increased to 100mg over 6 weeks. Leucovorin, 10 mg per day was given throughout the 18-week study. Because of late morning dizziness, the dose was split and was administered twice daily. SOD1 levels, Appel ALS scores (AALS), MQOL, and ALSFRS were determined at weeks 6, 12 and 18.

Results: Lymphocyte SOD1 levels dropped to 40–50% below baseline levels. AALS score on admission to the study was 61; upon completion of the 18-week study the AALS score was 44, indicating improvement. There was no change in ALSFRS or MQOL.

Discussion and conclusions: Our results show that in a patient with SOD1 associated FALS, pyrimethamine was well tolerated and effectively reduced SOD1 levels in peripheral blood lymphocytes by almost 50%. The observed improvement in the AALS is encouraging but additional patients are needed.

P137 INHIBITION OF SOD1 PROTEIN EXPRESSION IN THE CELL BY PYRIMETHAMINE, AN ORALLY AVAILABLE SMALL MOLECULE

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Background: Reduction of Cu/Zn superoxide dismutase (SOD1) protein levels was proposed to ameliorate mutant SOD1 familial amyotrophic lateral sclerosis (fALS). In the G93A mouse, siRNA against SOD1 was able to prevent the development of fALS symptoms. Thus, SOD1 protein expression is a validated target for development of drugs to treat fALS.

Objectives: To characterize the pharmacological control of SOD1 expression and to identify lead compounds to reduce SOD1 levels in the cell.

Methods: An ELISA-based high throughput screen was developed to detect the effects of drug treatment on SOD1 protein levels in cultured cells. HeLa, HEK-293, or Neuro2A cells were plated at optimized density and dosed with screening libraries at 10 uM. Total protein levels were determined and normalized using a Bradford assay, and cytotoxicity was monitored. Compounds capable of inhibiting SOD1 by more than 50% were confirmed in ELISA, and an IC50 was determined using the same technique. Potent compounds were tested in a Western blot to determine their specificity for SOD1 versus actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Results: The antimalarial dihydrofolate reductase (DHFR) inhibitor pyrimethamine (PYR) was identified as active in the high throughput screen designed to identify compounds to decrease SOD1 protein levels in the HeLa cell. Pyrimethamine was approved for the treatment of malaria in 1953, and is also used for the treatment of cerebrotoxoplasmosis. ELISA assays to measure SOD1 protein levels confirmed activity with an IC50 of 2 uM in both HeLa and the N2A cell lines following treatment with PYR for 72 hours. Dose-related decreases in SOD1 following PYR treatment were also measured using a different antibody in a Western blot. PYR did not affect actin or GAPDH expression, demonstrating selectivity rather than a global protein synthesis inhibition. This activity was also observed in HEK-293 cells. A series of DHFR inhibitors did not decrease SOD1 levels *in vitro*, demonstrating that DHFR inhibition is not the relevant mechanism of PYR.

Discussion and conclusions: Pyrimethamine selectively decreases the levels SOD1 protein *in vitro* and is a promising lead. Cell lines of neuronal lineage such as Neuro2a cells responded to PYR, as well as HeLa cells. Alsgen is conducting a lead optimization program to remove side effects, such as DHFR inhibition, and improve potency and pharmacokinetics. Analogs of PYR that do not inhibit DHFR also exhibit potent activity to decrease SOD1 levels in the cell. Studies to better define the mechanism of action of this class of compounds are also underway.

P138 PASSIVE IMMUNIZATION OF G93A MICE WITH MONOCLONAL ANTIBODIES SELECTIVE FOR MISFOLDED SUPEROXIDE DISMUTASE 1

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Background: Familial ALS can be caused by mutations in the gene encoding superoxide dismutase 1 (SOD1). Experiments performed in cell culture and mice transgenic for human mutant SOD1 have established that motor neurons are particularly vulnerable to SOD1 aggregation, some of which is secreted from neighbouring cells (1). Immunization of SOD1 mutant mice with whole mutant SOD1 can ameliorate this experimental disease (2). However, in order to avoid autoimmune complications, it is desirable to direct immune response against SOD1 peptides specifically exposed by misfolding in disease. This disease-specific epitope (DSE) immunotherapy concept was pioneered for prion protein (3) and recently extended to SOD1 (4).

Objectives: We sought to determine if SOD1-DSE immunotherapy of SOD1 mutant mice might impact on disease onset and progression.

Methods: SOD1 DSE peptide sequences were predicted to be exposed and unstructured during misfolding or metal depletion. Monoclonal antibodies (mAbs) directed against SOD1 DSEs were validated by selective reactivity against misfolded but not native SOD1. Candidate mAbs were tested in G93A mice by intracerebroventricular (ICV) infusion and intraperitoneal (IP) injection. Mice were

monitored by gait analysis using DigiGait as well as measuring clinical features. Endpoints were defined as weight loss >15%, and inability to right when placed on side.

Results: A series of immunological epitopes were identified which uniquely present on the molecular surface of misfolded/aggregated SOD1, but not the natively structured isoform. In particular, 3 mAbs reactive against the SOD1 electrostatic loop (designated DSE2) showed selective reactivity to SOD1 which was misfolded by denaturation and/or oxidation *in vitro*. Immunohistochemistry with these DSE2 mAbs showed neural SOD1 deposits in familial and sporadic ALS, as well as in G93A transgenic mice. ICV and IP treatment with DSE2 mAbs statistically significantly ameliorated signs of motor neuron disease in G93A mice.

Discussion and conclusions: A SOD1 DSE immune response can selectively target misfolded SOD1, while sparing natively structured SOD1 from autoimmune consequences. SOD1 DSEs are promising candidates for vaccination or immunotherapy of human ALS by blocking the propagation of SOD1 misfolding, and/or by immunological clearing of toxic SOD1 aggregates.

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P139 AN IMMUNIZATION STRATEGY FOR TREATING AMYOTROPHIC LATERAL SCLEROSIS THAT TARGETS MISFOLDED SOD1

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Background: Immunotherapy is emerging as a therapeutic approach in neurodegenerative diseases characterized by deposition of aggregated and/or misfolded proteins, including amyotrophic lateral sclerosis (ALS). In familial ALS (fALS1) cases harboring mutations within the gene encoding superoxide dismutase-1 (SOD1), SOD1 aggregates are found in motor neurons and in astrocytes. Although the precise molecular mechanisms of motor neuron degeneration caused by mutant SOD1 remains to be elucidated, it is suggested that toxicity is related to the propensity of the mutant protein to misfold and/or to dissociate from a dimer into a monomer, and ultimately to form aggregates. We have recently developed an antibody that recognizes monomeric/misfolded SOD1 called SOD1-Exposed-Dimer-Interface (SEDI) antibody. This antibody was generated to a peptide sequence corresponding to the dimeric interface of SOD1 (SEDI peptide) and therefore only detects SOD1 when this interface is exposed. Using the SEDI antibody, we have detected the presence of monomeric/misfolded SOD1 in motor neurons of three lines of mutant SOD1 transgenic mice (G93A, G37R and G85R) and fALS cases carrying SOD1 mutations.

Objectives: To assess the therapeutic potential of an active immunization strategy that selectively targets disease-associated protein species, monomeric/misfolded SOD1 or SOD1 aggregates.

Methods: Transgenic $SOD1^{G37R}$ were immunized intraperitoneally with SEDI peptide or mutant SOD1 pre-aggregated in vitro starting at six weeks of age. Titre boosts were made at two weeks post initial prime and once monthly for the next three months. Antibody titres and isotypes were assayed by enzyme-linked immunosorbent assays in serum samples collected over the immunization protocol. Mice were monitored for disease onset, progression and survival. Neuromuscular function and pathological outcomes were evaluated.

Results: Vaccination of $SOD1^{G37R}$ mice transgenic mice with SEDI peptide or SOD1 aggregates delayed the onset and slowed the progression of disease. Immunization increased life expectancy, improved motor deficits, and reduced motor neuron degeneration.

Conclusions: These results suggest that immunotherapy against pathological monomeric/misfolded SOD1 or SOD1 aggregates effectively improves motor and pathological disease outcomes. An immunotherapeutic approach that specifically targets misfolded SOD1 therefore provides promise for development of a novel therapy for the treatment of fALS1.

P140 SPINAL CORD ENGRAFTMENT OF GLUTAMATE TRANSPORTER OVEREXPRESSING ASTROGLIAL PROGENITORS FOCALLY PROTECTS RESPIRATORY FUNCTION IN A RAT MODEL OF ALS

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Background: Studies in ALS models have suggested that cellular abnormalities are not limited to motor neurons and that non-neuronal cells play a role in disease onset and progression. Glutamate excitotoxicity is one pathway that has been demonstrated to play a role in both ALS and in animal models of the disease. Compromised glutamate transport, due to decreased levels and aberrant functioning of the primary CNS glutamate transporter, GLT-1, occurs

in spinal cord astrocytes of both humans with ALS and in $SOD1^{G93A}$ rodents. Previous ALS stem cell transplantation studies have focused mostly on motor neuron replacement; however, clinical implementation of such an approach presents a number of challenges. Replacement of astrocytes may represent a more technically and biologically feasible approach in ALS patients. Cell transplantation into the cervical spinal cord specifically targets the region where motor neurons that control respiration reside. Since most ALS patients die of respiratory muscle paralysis, this novel approach has important clinical relevance for ALS therapy. Given these observations and other findings of astrocyte dysfunction in ALS, this proposal aims to target the replacement of dysfunctional astrocytes using glial restricted precursors (GRPs) for possible therapeutic benefits.

Objectives: 1. To determine the fate and survival of wild-type glial restricted precursors (GRPs) and GLT-1 glutamate transporter over-expressing GRPs (G3s) following transplantation into the $SOD1^{G93A}$ rat model of ALS. 2. To determine the capacity for motor neuron protection and assessment of the important physiological properties of glial precursors following transplantation. 3. To determine the ability of wildtype GRPs and G3s to preserve forelimb strength, diaphragmatic function, and survival following transplantation into the $SOD1^{G93A}$ rat.

Methods: GRPs and GLT-1 glutamate transporter over-expressing GRPs (G3s) were transplanted into the ventral horn of the cervical spinal cords of presymptomatic $SOD1^{G93A}$ rats at 3 levels (C4, C5, C6).

Results: The transplantation of glutamate transporter overexpressing GRPs results in differentiation into mature astrocytes which reside in the ventral gray matter adjacent to motor neuron soma and processes. $SOD1^{G93A}$ rats transplanted with GRPs demonstrate a preservation of diaphragm function, a slowing of forelimb grip strength decline and a delay in the onset of forelimb weakness. The effect was focal with no change in hindlimb grip strength. Survival was prolonged in glial progenitor transplanted $SOD1^{G93A}$ rats from the maintenance of respiratory function.

Discussion and conclusions: The transplantation of astroglial progenitors targeting specific ALS related pathways of neurodegeneration—specifically glutamate, results in the focal maintenance of respiratory physiology and function, a focal maintenance of forelimb strength and a slowing of the course of disease progression in the $SOD1^{G93A}$ rat. These improvements in function results in an improvement in survival and may offer a novel approach for stem cell replacement strategies in ALS patients with a focus on the preservation of respiratory function.

P141 BONE MARROW CELLS AS DELIVERY VEHICLES: GENE THERAPY IN A MOUSE MODEL OF ALS

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Background: Previous studies indicate that there is an increased flux of transplanted bone marrow (BM)-derived cells that enter the spinal cord in mice that over-express human mutant superoxide dismutase 1 (mSOD), a murine model of amyotrophic lateral sclerosis (ALS), compared to wild-type (wt) controls. BM-derived cells are readily accessible, and are capable of being maintained and stably transduced *in vitro.* This suggests that BM-derived cells may be ideal candidates for genetic manipulation and gene delivery into the central nervous system (CNS).
Numerous neurotrophic factors (NFs) have been shown to protect motor neurons, such as: brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF). Treatments utilizing NFs in patients and/or animal models of disease have mainly relied on oral, subcutaneous, or intrathecal administration, which have resulted in unsuccessful outcomes. More recently, viral-mediated gene therapies have been examined in mice and are showing more promising results.

Objectives: To use BM-derived cells as vehicles to transport NF genes across the blood-brain barrier (BBB) and into the CNS to decrease motor neuron degeneration and increase neuronal survival in a murine model of ALS.

Methods: BM cells were transduced *ex vivo* with lentiviruses that over-expressed genes encoding NFs: BDNF, GDNF, or VEGF. An IRES-GFP marker allowed for convenient tracking of positively infected cells both *in vitro* and in peripheral blood and CNS of BM-transplanted recipients. mSOD and wt mice were lethally irradiated and transplanted with NF-expressing BM.

Results: Mouse BDNF, GDNF, and VEGF genes have been isolated and cloned into a lentiviral vector. HEK-293T cells were transiently transfected (with an efficiency of $>90\%$) and viral-containing supernatant was used to infect NIH-3T3 cells (also with an efficiency of $>90\%$). 3T3 cells and cell supernatant were used to assess NF production and bioactivity. Either SDS-PAGE and Western blot analysis (GDNF, BDNF) or ELISA (VEGF) verified presence of the protein of interest. Bioactivity of the secreted proteins was demonstrated using dorsal root ganglion-outgrowth assays.

Discussion and conclusions: Because transplanted BM-derived cells are capable of crossing the Blood Brain Barrier (BBB), we have employed BM cells as delivery vehicles to transport NF genes locally to areas of the CNS affected by ALS. Current results indicate that high efficiency transient transfection of viral producing 293T cells and infection of 3T3 cells and BM can be obtained.

However, to be clinically useful we need to confirm stable long-term expression of NF genes by BM cells *in vivo*.

P142 GENETIC FUSION GDNF-TTC AND TTC DELAY DISEASE PROGRESSION AND PROLONG SURVIVAL IN SOD-1^{G93A} MICE

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Background: The successful use of glial cell-line derived neurotrophic factor (GDNF) to avoid neurodegeneration in animal models led to a clinical trial for treating ALS. However, these trials failed, probably due to the route of administration, as well as the poor bioavailability of this growth factor to target cells. Therefore, new administration routes for GDNF should be developed to solve this problem. Gene therapy vectors that can be targeted to motor neurons are required in this field. Different authors have proposed the C-fragment of tetanus toxin (TTC) as an efficient retrograde carrier from the skeletal muscle into neurons of the CNS. In previous studies, we show that TTC works as an enzymatic activity carrier to the CNS when muscle cells are transfected *in vivo* (naked DNA injection). A putative therapeutic strategy to reach specifically motor neurons could be achieved by the fusion of GDNF and TTC (GDNF-TTC).

Objectives: The aim of this study was to assess the effect on survival and disease progression of the naked DNA coding for GDNF-TTC after intramuscular injection in SOD-1^{G93A} mice.

Methods: Site-directed mutagenesis was performed to construct two different plasmids based on pcDNA3.1: CMV-GDNF-TTC and CMV-TTC. Plasmids were produced and extracted with EndoFree Plasmid Maxi Kit (Sigma) and diluted in PBS. Naked plasmids encoding for GDNF-TTC hybrid protein, TTC and control (empty plasmid) were used to transfect muscle cells in SOD-1^{G93A} mice**.** A unique dose of each plasmid was injected into four limbs (100ug in hindlimbs and 50 ug in forelimbs) of SOD-1^{G93A} mice at 8 weeks of age, an early symptomatic stage. Hanging wire test was performed to assess muscular strength and a rotarod device was used to determine motor function. Both tests were weekly performed and began at 8 weeks of age.

Results and Discussion: Expression of GDNF-TTC and TTC was confirmed by real-time PCR in muscle, 10 days after intramuscular injection of naked DNA. The onset of motor function deficits was observed at 14 week of age in control mice, as assessed by a decline in limb function in rotarod and hanging wire test. However, both GDNF-TTC and TTC treated animals significantly delayed the disease-induced onset of symptoms by two weeks. Furthermore, those mice treated with GDNF-TTC and TTC showed a lifespan prolonged by approximately 10 %. We have shown that the intramuscular injection of naked plasmid coding for GDNF-TTC provided significant motor function benefits and increased survival in SOD-1^{G93A} mice. Moreover, surprisingly our results showed that naked plasmid coding for TTC intramuscular injection yields similar effects.

Conclusion: These results suggest that non-viral gene therapy using TTC or GDNF-TTC provides a potential therapy for ALS treatment. Further work is being carried out to complete these data and to study the molecular mechanisms involved in these processes.

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P143 INTRAVENTRICULAR DELIVERY OF NEUROTROPHIC EXPRESSING AAV VECTORS PROVIDES SIGNIFICANT THERAPEUTIC BENEFIT IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by a selective loss of motor neurons in the motor cortex, brain stem and spinal cord. Currently there is no effective therapy for the treatment of ALS. Although a number of studies have demonstrated that neurotrophic factors have potent effects on motor neuron survival their delivery to the CNS remains a challenge. Recently it has been reported that AAV serotype 4 has an enhanced affinity for different cell types of the ventricular system including the ependymal cell layer and choroid plexus.

Objective: In this study we wished to determine if neurotrophic factor gene delivery to the ventricular system would be an effective strategy for delivering neurotrophic factors to the CNS of ALS mice in order to modify disease progression. Specifically, we evaluated the efficacy of intraventricular delivery of AAV4–IGF-1 and AAV4-VEGF in 90 day old (i.e., symptomatic) SOD1^{G93A} mice.

Methods: Starting at 80 days of age (and every 10 days thereafter), SOD1^{G93A} mice underwent behavioral testing (rotarod, hindlimb and forelimb grip strength) to assess motor function. At 90 days of age (i.e., time point at which SOD1 mice exhibit overt disease symptoms of ALS) mice received intraventricular injection of AAV4-IGF-I or AAV4-VEGF or AAV4-GFP.

Results: We found that delivery of neurotrophic expressing AAV4 vectors (regardless of transgene) significantly improved motor performance in both rotarod and grip strength behavioral tests and significantly extended lifespan (i.e., 20 days). Treatment with AAV4-GFP had no effect on any of the parameters mentioned above.

Conclusion: Our results indicate that intraventricular injection of neurotrophic expressing AAV4 vectors is an effective approach for modifying disease progression in a mouse model of ALS.

P144 INTRAVENOUS NON-VIRAL GENE TRANSFER OF IGF-1 SHOWS THERAPEUTIC BENEFIT FOR SOD1 MOUSE MODEL OF ALS

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Background: Motor neuron degeneration in animal models for amyotrophic lateral sclerosis (ALS) can be mitigated by neurotrophic factors, which are therefore considered as good candidates for treatment of human ALS. A number of neurotrophic factors (e.g., VEGF, GDNF and IGF-1) have been demonstrated to promote survival and/or regeneration of motor neurons both *in vitro* and *in vivo*. However, the delivery of these factors for therapeutic purpose is problematic because of their short half-life and poor penetration into the central nervous system (CNS) through the blood brain barrier. A form of gene transfer and sustained expression of neurotrophic factors may overcome these problems. Previously, an increase in the lifespan of transgenic mutant superoxide dismutase-1 (SOD1/G93A) mice was demonstrated following injections of glial-cell derived neurotrophic factor (GDNF) or insulin-like growth factor (IGF-1) expressing viral vectors. For human gene therapy, a non-viral vector has significant advantages over viral vectors because of a better safety profile and a lower cost of vector production.

Objective: To determine if mouse IGF-1 gene transfer into skeletal muscles alters the clinical course of SOD1 mice when delivered intravenously.

Methods: A mouse IGF-1 cDNA expression plasmid was constructed and three different doses (30, 100, 300 μg/limb) were administered into genotyped SOD1 mice at 80–90 days of age after the onset of motor dysfunction. ''Naked'' plasmid DNA was administered by hydrodynamic delivery into the bilateral great-saphenous veins of the hind limbs.

Results: SOD1 mice treated with mIGF-1 lived significantly longer (14 days for 30 μg; 19 days for 100 μg and 29 days for 300 μg plasmid DNA per limb) than untreated SOD1 mice. This improvement in survival was equal if not better to that observed with adeno-associated virus vectors. IGF-1 expression was detected in limb muscles and the levels corresponded to the injected plasmid DNA doses. Motor performance (measured by RotaRod) declined less rapidly in the IGF-1 treated SOD1 mice than in the controls and the best performance was observed in the group that was injected with the highest dose of plasmid. Compound muscle action potential amplitudes (an electrophysiological parameter of motor unit integrity) were significantly higher in SOD1 mice treated with IGF-1 gene transfer. We showed a retrograde transport of tagged IGF-1 protein to a large number of anterior horn cells.

Conclusions: These data suggest that plasmid DNA-mediated, intravenous gene transfer of mouse IGF-1 had a beneficial effect for SOD1 mice in both survival and motor functions. It is most likely that this effect was due to target-derived action of IGF-1 originating from muscle over-expression. This gene therapy technique is promising for the treatment of ALS.

P145 MYOGANE™, AN ORAL NEUROTROPHIC FACTOR INDUCER, DELAYS THE LOSS OF MUSCLE STRENGTH AND EXTENDS SURVIVAL TIME IN TWO *IN VIVO* MODELS OF MOTOR NEURON DISEASE

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Background: The precise causes of motor neuron degeneration in ALS patients remain unknown. Possible mechanisms include loss of neurotrophic factors coupled with oxidative and glutamate mediated damage of nerve cells. Neurotrophic factors are essential for the survival and maintenance of nerve cells however as proteins, their utility as pharmacological treatments is limited. An animal model of ALS that exactly correlates the human disease does not exist; consequently we have used two models of MND.

Objectives: To examine the effects of Myogane™ (PYM50018), an oral neurotrophic factor inducer, on two models of MND: (1) the SOD1-G93A transgenic mouse over-expressing the mutant human Cu, Zn SOD SOD1-G93A (displaying spinal motor neurone degeneration and alterations in cytoskeletal components) (2) the progressive motor neuropathy (*pmn*) mutant mouse (displaying a progressive ''dying-back'' type degeneration).

Methods: This study investigated the effects of Myogane™ administered daily (*p.o.*), to SOD1-G93A and *pmn* mice following the onset of the clinical symptoms, on electrophysiological parameters (compound muscle action potential, CMAP: amplitude), behavioural parameters (grid test) and survival profile. Electrophysiological and behavioural results were analysed by a multivariate

analysis of variance. Survival rates were evaluated by the Kaplan-Meier method. Significance was taken as $p < 0.05$.

Results: Administration of Myogane™ (0.3 mg/kg/day, n=6) to SOD1-G93A mice significantly delayed the onset of the muscle strength loss compared with vehicle treated SOD1-G93A mice (CMAP: $p < 0.001$; grid test $p < 0.01$) and significantly increased the life span of SOD1 mice by 11% ($p < 0.05$). Administration of Myogane™ (0.3 μg/kg/day; n=6) to *pmn* mice significantly delayed the onset of the muscle strength loss compared with vehicle treated *pmn* mice (CMAP: $p < 0.001$; grid test; $p < 0.05$) and significantly increased the life span of *pmn* mice by 62% ($p < 0.01$).

Discussion and conclusions: Myogane™, an oral non-peptide neurotrophic factor inducer, currently in clinical development, delays the loss of muscle strength and extends the lifespan in two different animal models of MND. These results suggest that Myogane™ is a promising candidate for the treatment of MND.

P146 SLOWING THE PROGRESSION OF NEURONAL DEGENERATION IN ALS MOUSE MODEL USING NAIP-UPREGULATING COMPOUNDS THAT SELECTIVELY INHIBIT OXIDATIVE STRESS-INDUCED CELL DEATH

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Background: Oxidative stress is known to be one of major factors implicated in the onset and progression of several types of neurodegenerative diseases including ALS/MNDs. Neuronal apoptosis inhibitory protein (NAIP, aka BIRC1) is a member of the BIR-domain containing inhibitor of apoptosis protein (IAP) family, and selectively inhibits oxidative stress (and/or reactive oxygen spices) induced cell death. Ectopic NAIP expression rescues motor neurons after peripheral nerve axotomy, and hippocampus CA1 neurons in bilateral common carotid artery occlusion mouse model. Endogenous NAIP up-regulation reduces neuron damage/death in cerebral ischemia. Hence, NAIP is thought to be a potent molecular target in ALS/MND drug discovery. It is conceivable that upregulation and/or stabilization of endogenous NAIP could protect against neurodegeneration that is associated with oxidative stress. Thus, identification of NAIP-upregulating compounds will provide a novel therapeutic means towards the treatment and/or cure of ALS/MNDs.

Objectives: The purpose of this study was to identify NAIP-upregulating compounds, and to investigate their drug efficacy *in vivo* using a *SOD1*-H46R ALS mouse model, thereby proving a preclinical proof-of-concept for NAIP-based drug development in ALS/MNDs.

Methods: The NAIP-double sandwich-ELISA system in conjunction with *in vitro* cell cultures, which we have recently established, was used for the identification of the NAIP-upregulating compounds. The identified compounds were then subjected to cell viability assay, flow-cytometric analysis, and Western blotting to confirm their protective competence against oxidative stress-induced cell death. Next, NAIP-upregulating compound efficacy was tested in an ALS (*SOD1*-H46R) mouse model using oral daily administration commencing either pre- or post-onset. Behavioral (balance-beam) and survival (Kaplan-Meier) analyses were performed.

Results: L-745,870, dopamine D4 receptor antagonist, was obtained through NAIP-double sandwich-ELISA and in vitro assessment with 935 neurotropic compounds. L-745,870 including other hit compounds selectively upregulated NAIP and revealed selective protection against oxidative stress-induced cell death. L-745,870 (10mg/kg/day), pre-onset administration, delayed onset (5.3 days) and slowed progression in ALS (*SOD1*-H46R) mice. Post-onset administration of the compound remarkably prolonged survival after the onset compared to the naive mice (47.6 ± 7.3 days vs. 36.5 ± 11.0 days, $p < 0.05$), indicating that L-745,870 as well as other NAIP up-regulating compounds have a therapeutic potential in ALS.

Conclusion: In this study, we demonstrated that the NAIP-upregulating compound, L-745,870, delayed onset and prolonged life-span after the onset (slowing progression) in an ALS (*SOD1*-H46R) mouse model. Most importantly, the post-onset administration of the compound resulted in a significant slowing of disease progression in the ALS mouse model. Thus, our studies provide a preclinical proof-of-concept; the therapeutic feasibility for ALS/MNDs. Future studies with the NAIP-based drug screening in conjunction with in silico drug screening and drug design will identify effective compounds for the treatment of ALS/MNDs.

P147 TREATMENT WITH ARIMOCLOMOL PREVENTS MORPHOLOGICAL AND FUNCTIONAL CHANGES AT THE NMJ OF SOD1^{G93A} MICE

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Background: Distal changes at the neuromuscular junction (NMJ) have been shown to precede motoneuron degeneration in SOD1^{G93A} mice and in post-mortem ALS muscle (1) We have previously shown that treatment with arimoclomol, a co-inducer of the heat shock response, prevents motoneuron degeneration and extends survival of SOD1^{G93A} mice (2). Arimoclomol therefore has clear

neuroprotective effects on motoneurons within the CNS. Whether arimoclomol also has effects in the periphery at the NMJ has not been previously investigated.

Objectives: In this study we examined the effect arimoclomol on the rate of denervation of hindlimb muscles in SOD1^{G93A} mice and correlated these findings with an assessment of markers of functional neuromuscular transmission.

Methods: SOD1^{G93A} mice were treated daily from 35 days with 10 mg/kg arimoclomol (i.p) and assessed at 45, 75, 90 and 120 days of age. The level of innervation of slow (soleus) and fast (extensor digitorum longus, EDL) muscles in treated and untreated SOD1^{G93A} mice was established morphologically using a quantitative histochemical silver-cholinesterase stain. The extent of denervation at each age was correlated with changes in functional markers of neuromuscular transmission by examination of the biochemical activities of the cholinergic enzymes acetyl cholinesterase (AChE) and choline acetyltransferase (ChAT) in muscles, axons and spinal cords.

Results: As has been shown by others, slow and fast muscles are differentially affected during disease in SOD1^{G93A} mice. The fast EDL was more vulnerable and became denervated earlier than the slow soleus muscle. However, the greatest loss of innervation in EDL occured after disease onset. Furthermore, in both EDL and soleus muscles as well as nervous tissue, AChE activity declined prior to the loss in ChAT activity. Following treatment with arimoclomol, there was a significant improvement in the level of innervation of both EDL and soleus muscles in symptomatic mice. However, the most dramatic effect of arimoclomol was a complete restoration of the activity of both AChE and ChAT to normal levels, reversing the reduction observed in untreated mice.

Discussion: The results show that in addition to preventing motoneuron degeneration within the spinal cord, arimoclomol prevents many of the deleterious effects that occur at the NMJ prior to neuronal loss. These effects of arimoclomol at the NMJ may reflect the known actions of arimoclomol on motoneurons and astroglia in the CNS. It is therefore possible that events that occur at the NMJ of SOD1^{G93A} mice prior to motoneuron degeneration may be the result of other changes occurring within the cell body of the motoneuron. Alternatively, our findings may reveal direct peripheral effects of arimoclomol at the NMJ, which are independent to its effects on spinal cord motoneurons and astroglia.

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P148 ARIMOCLOMOL MODIFIES THE EXPRESSION OF CO-CHAPERONES AND NEUROINFLAMMATORY MARKERS IN THE SOD1^{G93A} MOUSE MODEL OF ALS

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Background: Although the precise pathogenesis of ALS remains unclear, recent results demonstrate that it is a non-cell autonomous disease in which a number of cell types including motoneurons, astroglia and microglia all contribute to motoneuron degeneration. Evidence also suggests that depletion of members of the endogenous protective heat shock response may also play a role in ALS. We have previously shown that treatment with arimoclomol, a co-inducer of the heat shock response, protects motoneurons from cell death and extends lifespan in the SOD1^{G93A} mice, by up-regulating the expression of heat shock proteins (hsps).

Objectives: In this study we investigated the effects of treatment of SOD1^{G93A} mice with arimoclomol, on the expression of co-chaperones, members of the heat shock response that assist in hsp function such as protein refolding and proteasome sorting. We also examined the effect of arimoclomol on the expression of neuroinflammatory markers, such as GFAP and COX-2 that are upregulated in ALS during disease progression.

Methods: SOD1^{G93A} mice were treated daily from 35 days with 10 mg/kg arimoclomol (i.p) and assessed at 120 days of age when previous results have shown an upregulation in hsp expression and an increase in motoneuron survival and muscle function. The expression of co-chaperones and inflammatory markers in spinal cord of treated and untreated SOD1^{G93A} mice was examined by immunocytochemical and Western blot analysis. The pattern of expression of these markers in motoneurons and glial cells was determined.

Results: Our results show that treatment with arimoclomol altered the expression of a number of co-chaperones such as p23 and CHIP in different cell populations within the spinal cord. Arimoclomol not only modified co-chaperone expression, but also altered the distribution of their expression within different cell types. Furthermore, there was a marked decrease in astrogliosis in arimoclomol-treated mice, as indicated by reduced expression of GFAP.

Discussion: Pharmacological modification of the heat shock response by treatment with arimoclomol not only affects protein folding and degradation but may also ameliorate other pathological hallmarks of ALS, such as neuroinflammation. These diverse effects of arimoclomol may contribute to its neuroprotective actions and its ability to significantly delay motoneuron degeneration in SOD1^{G93A} mice.

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THEME 7 IN VIVO EXPERIMENTAL MODELS

P149 A COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF GASTROCNEMIUS AND TRICEPS MUSCLES IN AN ALS MOUSE MODEL

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Background: Amyotrophic lateral sclerosis (ALS) is a degenerative disease which leads to the progressive and selective degeneration of cortical and spinal motor neurons. Beyond this main neuropathological feature, alterations in the morphology and metabolism of skeletal muscle have also been identified both in animal models and in patients affected by the disease. However, muscle involvement in the pathogenesis of ALS is still unclear. Muscular over-expression of trophic factors such as IGF-1 or GDNF in $SOD1^{mut}$ transgenic mice delays the onset of the disease and exerts a neuroprotective effect on affected motor neurons. On the contrary, it has been shown that muscle-specific silencing of mutant SOD1 affects neither the onset of the disease nor the survival of the transgenic mice. Despite these contrasting results, the analysis of the muscle tissue is of great interest as it may reveal important clues to the understanding of the pathogenetic mechanisms of ALS.

Materials and Methods: In this study we have characterized the gene expression profile of two different muscles from transgenic $SOD1^{G93A}$ and control mice at both non-symptomatic and symptomatic stages of the disease by the use of Affymetrix Genechip technology. Gastrocnemius and triceps muscles were considered since the first is severely affected at early stages of the disease, while the second is involved only at a later stage. To evaluate and to exclude from our data all gene expression changes associated to physiological denervation processes and muscular atrophy, we have also analysed the transcription profile of gastrocnemius after crushing the sciatic nerve.

Results: Gene Ontology analysis of the differentially expressed genes at both ages revealed a partial overlap between the transcription profiles of the two muscles, although some differences are evident. We could discriminate between gene expression changes due to aging and denervation and those associated with the disease process. In particular, at non-symptomatic stages of the disease, few changes in gene expression profile were observed in either muscle. On the contrary, at a symptomatic stage, the two muscles show a different involvement in the disease. Triceps muscle seems to be still engaged in regenerative processes, while gastrocnemius is already atrophic with a predominant activation of the apoptotic pathway.

Discussion: Our data support the importance of skeletal muscle for the search of biological markers of the disease since it recapitulates the pathological processes that also lead to the degeneration of motor neurons.

P150 GENE EXPRESSION PROFILING TO INVESTIGATE THE STRESS EFFECTS OF PHYSICAL EXERCISE ON THE MOTOR NEURONE TRANSCRIPTOME

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Background: Well known sportsmen such as Lou Gehrig (baseball), Ezzard Charles (boxing), Donald Levey (football) and Jarrod Cunningham (rugby) have developed MND, leading to the assessment of physical activity as a risk factor for the disease. In several studies, participation in sports/athleticism has been identified as a risk factor, although there has not been universal agreement with this conclusion. In addition, recent reports have shown an increased incidence of ALS in Italian soccer players. Previous studies have identified genes differentially expressed in response to exercise in muscle, blood and the hippocampus. However, no studies have investigated genes differentially expressed in MN, the vulnerable cell population in MND, in response to this stress.

Objective: Our aim is to identify 1) which genes are activated/repressed in response to exercise in muscles and MN in absence of other stress; 2) which genes belong to a specific motor neuronal response; 3) whether there may be any relationship between the cellular stress response to physical exercise and that associated with the presence of mutant SOD1.

Methods: Gastrocnemius muscle and approximately 1000 motor neurons have been isolated from lumbar spinal cord of 3 mice undergoing voluntary exercise (mean running distance of 10km per day) for 21 ± 1 days and 3 sedentary mice. Muscular RNA was isolated using Quiazol kit (Qiagen); motor neuronal RNA was extracted using Picopure kit (Arcturus), amplified using the Affymetrix Amplification kit (Arcturus) and labelled using the GeneChip Expression IVT Labelling Kit (Affymetrix). 10μg cRNA was applied to the Affy Mouse Genome 430

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2.0 GeneChip, and data analysis was performed using ArrayAssist (Iobion).

Results: The comparison between the transcriptome of motor neurons isolated from exercised and sedentary mice shows significant changes in 1447 genes (fold change $\geqslant$1.5, P value $\leqslant$0.05). 144 out of 1447 are upregulated in the exercised mice, 1303 are downregulated. They have been categorised by function and analysed using pathway analysis programmes. The downregulated genes belong to several different classes, including, surprisingly, signalling pathways and carbohydrate metabolism. A thorough analysis of the upregulated genes identified the upregulation of specific pathways such as phosphatidylinositol-3 kinase pathway, serine metabolism and related neurotransmitters (D-serine and glycine as co-agonists of NMDA receptors). The comparison between the transcriptome of muscles extracted from exercised mice and sedentary mice shows significant changes in 362 genes (fold change $\geqslant$1.5, P value $\leqslant$0.05). 67 of the 362 altered transcripts are downregulated. Our study shows clear signs of increased angiogenesis and matrix reorganization, as supported by the activation of several proteins involved in these processes (e.g. periostin, osteoclastin, collagens, cadherin 5 and matrix metallopeptidase 2). Exercised muscles show a massive upregulation of the immune response, involving activation of the complement, of the histocompatibility complex class 2 (MHC2), several chemokines and P lysozyme.

Discussion: The transcriptional response of MN to exercise differs from that caused by the presence of the mutant SOD1 protein. Nevertheless we observed the involvement of common key pathways, e.g. carbohydrate and lipid metabolism and glutamate related neurotransmission.

P151 EXPRESSION OF FMO1, FMO2, FMO3, FMO4 AND FMO5 GENES IN TRANSGENIC MOUSE MODELS OF ALS

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Background: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset, progressive and fatal neurodegenerative disease of unknown pathogenesis. Motor neuronal degeneration represents the main pathological feature in ALS, for which two major pathogenetic hypotheses have been proposed: oxidative stress and excitotoxicity. Flavin-containing monooxygenases (FMO) represent a gene family coding for microsomal enzymes which are involved in the oxidative metabolism of a variety of xenobiotics. This family contains five genes, FMO1, FMO2, FMO3, FMO4, FMO5, and at least six pseudogenes in humans. The function of FMO genes has largely been studied in liver, kidney and lung but not in nervous system. Recently, the observation of a 80% reduction in FMO1 mRNA expression in spinal cord of sporadic ALS patients pointed to a relationship between ALS and FMO genes. In addition, in a previous study, we found an association between two 3′-UTR single nucleotide polymorphisms of FMO1 gene and the development of sporadic ALS.

Objectives: To investigate the mRNA levels of FMO1, FMO2, FMO3, FMO4, FMO5 in murine nervous system in normal mice and in a transgenic murine model of ALS.

Methods: The C57BL/6J mouse strain with the human-SOD1 G93A mutation was used as the disease model; C57BL/6J normal mice were used as controls. Four brain areas were examined in all animals: cerebellum, cerebral hemispheres, brainstem and spinal cord. Real-Time PCR technique with TaqMan probes was used for expression analysis. Normalization was optimized using Hprt as housekeeping gene.

Results: Our study demonstrates that all FMO genes, except for FMO3, are expressed in the murine nervous system. The FMO expression is sex-dependent and varies over different brain areas. Moreover, alterations in FMO expression have been found in G93A mutated mice. Particularly, in G93A males, we observed in different cerebral areas a lower expression of FMO2 and FMO4 gene compared to WT mice. Conversely, G93A female mice expressed significantly greater amounts of FMO2 and FMO5 genes in the cerebellum and cerebral hemispheres.

Discussion and conclusions: Our study represents the first attempt to analyse FMO gene expression in different areas of the nervous system and particularly in the spinal cord. We confirmed the sex-dependent expression of FMO genes in nervous system, as already found in other tissues. The finding of altered FMO expression in a model of motor neuron degeneration might suggest a role for FMO genes in the development of ALS, even if the pathogenic link is presently unknown.

P152 SELECTIVE COMPENSATORY INCREASE IN PRESYNAPTIC TERRITORY ON LUMBAR MOTONEURONES BY A PROPRIOSPINAL SYNAPSE IN A MOUSE MODEL OF MND/ALS

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Background: Physiological evidence in muscle and intracortical and corticostriatal pathways of patients, and general cytochemical evidence in muscle and spinal cords of mSOD1 mice indicates a capacity for compensatory synaptic plasticity in MND/ALS. Ultrastructural evidence in individual synaptic spinal cord pathways however is lacking.

Objective: To obtain such evidence in the lumbar spinal cord of G93A SOD1 mice.

Methods: Male *B6SJL-TgN(SOD1-G93A)1Gur* mice transgenic (Tg) and wild-type (Wt) littermates were

examined at ages 6 weeks, 10 weeks and 18 weeks. Anesthetised mice were perfusion-fixed with buffered 2.5% glutaraldehyde, and their spinal cords removed and processed for electron microscopy (EM). Individual lumbar MN were identified in 1um 'plastic' sections and re-identified by EM in adjacent ultrathin (70nm) sections. Presynaptic terminals of MN sectioned through a mid-nuclear plane were classified using prescribed ultrastructural criteria (1). Computerised morphometry (2) on images magnified x50K measured presynaptic terminal lengths and sizes of post-synaptic structures in samples of 20–30 MN per mouse.

Results: Mean onset of hindlimb paralysis Tg mice was 113d, mean survival was 127 ± 7.9d. Five ultrastructural classes of presynaptic terminal synapsed with MN (S, F, T, M, & C) in Wt mice. By 10 weeks, degeneration of MN and most classes of terminal occurred in Tg mice but by 18 weeks, C-type terminals on surviving MN appeared larger with more post-synaptic rER. Exclusive to alpha-MN, the C-type was characterised presynaptically by 50nm synaptic vesicles, and post-synaptically by an opposing subsynaptic ER cistern and underlying Nissl body (rER lamellae and polyribosome arrays). Morphometric analyses found no significant differences between C-terminal lengths or sizes of post-synaptic Nissl body in Wt and Tg mice at 6 weeks. Mean C-terminal length was 134% larger in Tg mice at 10 weeks (Wt/Tg difference significant at $p < 0.01$), and 160% larger at 18 weeks (significant at $p < 0.0004$). C-terminals exceeding 3 microns in length comprised 25% in Wt at all ages, but over 50% in Tg mice by 18 weeks, indicating para-terminal growth. Growth was matched by an increase in mean numbers of post-synaptic rER lamellae (124% at 10 weeks, 260% at 18 weeks, Tg/Wt difference $p < 0.0002$), with an increase in mean overall length of rER lamellae per terminal (151% at 10 weeks, 280% at 18 weeks, Tg/Wt difference $p < 0.0001$). Our studies also suggest paraterminal growth may be accompanied by increased numbers of C- terminals (ie 'sprouting').

Conclusions: Our data identify a novel compensatory and selective response by a morphologically distinctive axon terminal, comprising an increase in its presynaptic territory and enlargement of its associated post-synaptic Nissl body. In a wider context, C-terminals derive from short-axon intrasegmental cholinergic interneurones, and matched paraterminal growth and post-synaptic ribosomal proliferation characterises reactive synaptogenesis in other circumstances (2). Our results may indicate an attempt by one class of premotor interneurone to improve synaptic communication with surviving MN, and offer a means of assessing strategies for encouraging synaptic communication in models of MND/ALS.

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P153 DEATH OF MOTOR NEURONS IN MICE DEFICIENT IN AN RNA EDITING ENZYME

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Background: Deficient RNA editing of GluR2 mRNA at the Q/R site occurs specifically in motor neurons of sporadic ALS but not in those of other motor neuron diseases including ALS1 and SBMA, and has been proposed to be a possible cause of neuronal death in ALS motor neurons. This molecular change is likely due to a reduction in the activity of adenosine deaminase acting on RNA type 2 (ADAR2), an editing enzyme that specifically edits the GluR2 Q/R site (1).

Objectives: To elucidate whether a loss of ADAR2 induces death of motor neurons via reducing RNA editing of GluR2 mRNA at the Q/R site.

Methods: We have developed genetically modified mice in which ADAR2 gene is selectively knocked out in motor neurons using Cre-loxP system. We investigated the changes in motor function, survival, neuronal loss and RNA editing of motor neurons in these mice.

Results: These mutant mice displayed significantly shorter mean survival than control mice and progressive motor-selective behavioral deficit with delayed loss of spinal motor neurons. RNA editing at the GluR2 Q/R site was markedly decreased in motor neurons in these mutant mice.

Discussion and conclusions: Here we demonstrated that loss of ADAR2 induces under-editing of GluR2 mRNA at the Q/R site and results in the slowly progressive death of motor neurons in the ADAR2 knockout mice. Thus it is likely that GluR2 under-editing in motor neurons of sporadic ALS is the causative molecular change of neuronal death. Because the Q/R site of GluR2 mRNA is specifically edited by ADAR2, death of motor neurons in sporadic ALS may be due to a reduction of the ADAR2 activity. This mutant mouse model would be useful in investigating the aetiology of sporadic ALS.

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P154 D-SERINE EXACERBATES GLUTAMATE EXCITOTOXICITY IN ALS

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Bakground: Glutamate excitotoxicity mediated by ionotropic glutamate receptors has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). A more recent finding shed light on the relevance of the NMDARs in ALS pathogenesis: an NMDAR antagonist memantine has been reported to prolong survival of ALS model mice. The activation of the NMDARs essentially requires the binding of a co-agonist such as D-serine to its glycine site. Considering that insults generated from activated glial cells are assumed to be critical to the induction of motoneuronal death, we hypothesized that the excessive amounts of D-serine, generated from the activated glial cells, may contribute to the development of glutamate toxicity in ALS.

Objectives: This work was performed to demonstrate the contribution of an NMDA receptor co-agonist, D-serine, to motoneurons in ALS as a glia-derived enhancer of glutamate excitotoxicity.

Methods: We detected D-serine by immunohistochemistry and chemiluminescence assays. Production of serine racemase (SRR)/ degradation of D-amino acid oxidase was assayed by immunohistochemistry and Western blotting analysis using spinal cords from G93A-SOD1 transgenic mice and sporadic/familial ALS patients. **A** microglial cell line was used to demonstrate how D-serine production became excessive in ALS by detecting the expression level of SRR after an inflammatory factor treatment or infection of adenovirus carrying mutant SOD1. We performed cell death assay by measuring LDH release from primary spinal cord culture treated with NMDA/D-serine-related reagents.

Results: Levels of D-serine and SRR in spinal cords of ALS mice were progressively elevated, predominantly in microglia, with disease progression. *In vitro*, expression of serine racemase was induced not only by an extracellular pro-inflammatory factor but also by transiently expressed G93A-SOD1 in microglial cells. In primary cultured spinal cord cells, neurons from ALS mice were more vulnerable to NMDA-induced excitotoxicity compared with those from control mice. The toxicity to neurons from ALS mice was largely prevented by co-incubation with an inhibitor for serine racemase. Increases of D-serine levels were also observed in spinal cords of both familial and sporadic ALS patients.

Discussion and conclusions: We would like to propose a novel hypothesis to explain the mechanism underlying motoneuronal death involving glial activation in ALS pathogenesis. In this hypothesis, we think that the elevation of SRR expression, initially induced by glial activators and/or mutant SOD1, subsequently increases D-serine levels in activated glias. The elevated levels of D-serine in the extracellular space in turn augment Glu toxicity to motor neurons through NMDARs. These findings may provide a novel therapeutic target relevant to decreasing the level of D-serine or blocking D-serine binding site of NMDAR.

P155 LOSS OF ALS2/ALSIN EXACERBATES MOTOR DYSFUNCTION IN A MUTANT SOD1-EXPRESSING MOUSE ALS MODEL

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Background: *ALS2* mutations account for a number of juvenile motor neuron diseases (MNDs), including 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). Thus, its gene product ALS2/alsin may play an important role in maintenance and/or survival of motor neurons. Recently, it has been shown that overexpression of ALS2/alsin protects cultured motor neuronal cells from toxicity induced by mutant SOD1, suggesting a possible neuroprotective function of ALS2/alsin. We hypothesized that ALS2/alsin could modulate the onset and/or progression of ALS/MNDs.

Objectives: To examine whether ALS2/alsin plays a role in the onset and/or progression of MND that is associated with mutant SOD1 *in vivo*.

Methods: We generated $SOD1^{H46R}$ transgenic mice on an *Als2*-null background by crossing *Als2*-KO mice with the $SOD1^{H46R}$ mouse line expressing familial ALS-linked $SOD1^{H46R}$ under the control of inherent human *SOD1* promoter. Since genetic background is one of the important factors modulating disease phenotypes in mutant SOD1 transgenic mice, we first generated congenic lines of both $SOD1^{H46R}$ transgenic and *Als2*−/− mice by backcrossing each more than 10 generations with C57BL/6J mice. Next, we produced *Als2*+/−;$SOD1^{H46R}$ mice by crossing male $SOD1^{H46R}$ and female *Als2*+/− mice, and then generated six different genotype mice; wild-type, *Als2*+/−, *Als2*−/−, $SOD1^{H46R}$, *Als2*+/−;$SOD1^{H46R}$ and *Als2*−/−;$SOD1^{H46R}$, by crossing male *Als2*+/−;$SOD1^{H46R}$ and female *Als2*+/− mice. Body weight and survival of each animal were monitored. Motor co-ordination and balance were also measured by a balance-beam test to evaluate the motor dysfunction in the mice.

Results: As previously reported, *Als2*−/− mice did not show any gross abnormal phenotypes during the experimental period (~32 weeks). In contrast, $SOD1^{H46R}$ and *Als2*+/−;$SOD1^{H46R}$ mice both exhibited progressive motor dysfunction and paralysis with average life spans of 165.1 ± 10.3 (n=120) and 163.8 ± 10.1 days (n=107), respectively. Remarkably, *Als2*−/−;$SOD1^{H46R}$ mice showed a shorter life span of 152.4 ± 4.3 days (n=39).

Survival in *Als2*−/−;SOD1^{H46R} mice was significantly shorter than those in wild type or *Als2*+/−;SOD1^{H46R} mice ($p < 0.001$, Kaplan-Meier analysis with log-rank test). Further, a balance-beam test revealed that motor dysfunction in *Als2*−/−;SOD1^{H46R} mice (~14 weeks of age) occurs at an approximately 3 weeks earlier than that in *Als2*+/−;SOD1^{H46R} or SOD1^{H46R} mice (~17 weeks of age).

Discussion and conclusions: Our results suggest that loss of ALS2/alsin exacerbates motor dysfunction in SOD1^{H46R} mice. Preliminary biochemical characterizations revealed that the increased levels of insoluble mutant SOD1 were observed at earlier than 8 weeks of age in cortex and spinal cord of *Als2*−/−;SOD1^{H46R} mice but not of other genotype littermates, suggesting that ALS2/alsin deficiency may hinder protein degradation and/or accelerate the accumulation of the SOD1-containing aggregates in SOD1^{H46R} mice. Further characterization of these mice will clarify the implication of the ALS2/alsin-mediated functions in mutant SOD1-linked ALS *in vivo*.

P156 ALTERED LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) REVEALS EARLY PRE-SYMPTOMATIC MOTOR NEURON PATHOLOGY IN SOD1-G93A MICE

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Background: While loss of spinal motor neurons and deficits in motor tasks appear at around postnatal day (P) 90 of life in SOD1-G93A mice, structural changes at neuromuscular junctions have already been detected at P50 (1). No signs of early pre-symptomatic pathology at the site of motor neuron cell bodies, however, have been documented. Recently, we showed that CGRP-related immunoreactivity (IR) not only served as a marker for spinal motor neurons, but also labelled dystrophic dendrites and axons in the spinal cord ventral horn at P90 in SOD1 mice (2).

Objective: In the present report we investigated the onset and progression of CGRP-related neuropathology in the SOD1-G93A mouse model of ALS to test whether CGRP could serve as a novel early marker of spinal motor neuron pathology.

Methods: Lumbar spinal cord and skeletal muscle from SOD1 and wild type mice were analysed from P1 to P130 in 10 day intervals by immunohisto-chemistry for CGRP, ChAT, VAChT, MAP2, NF200, SOD1 and Ubiquitin. The mRNA expression patterns of the two CGRP isoforms (alpha and beta) were determined with *in situ* hybridization histochemistry. AlphaCGRP knockout mice were utilized to confirm the specificity and selectivity of CGRP antibodies and riboprobes.

Results: In wild type mice, CGRP-related IR was present in ventral horn motor neuron cell bodies and in dorsal horn sensory fibers. Starting at P40 in SOD1 mice, additional CGRP-related IR appeared in spherical structures in the motor neuron area. These structures increased in size from 2–3μm at P40 to $>5\mu$m at P100, and in number with a peak at around P80. CGRP-IR spherical structures were weakly present in motor neuron cell bodies and strongly in the neuropil around them. From P50 on, they were also detected by antibodies against Cu/Zn-SOD1 and ChAT, but lacked VAChT- and Ubiquitin-IR. MAP2 and NF200 co-labeling pointed to a localization in dendrites and axons, respectively. However, CGRP-related IR was absent from neuromuscular junctions, even at P20. CGRP-positive spherical structures were not detected by an alphaCGRP selective antiserum, and therefore most likely contained exclusively betaCGRP. In contrast to the differences in alpha- and betaCGRP-related IR patterns, alpha- and betaCGRP mRNA expression patterns were similar in SOD1 mice across all stages analysed.

Conclusion: Our data indicate that CGRP-related IR is a novel marker for early motor neuron pathology at the spinal level in the SOD1-G93A transgenic ALS mouse model, which uncovers that motor neuron degeneration already starts around P40.

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P157 LOCALIZATION OF O-GLYCOSYLATED PROTEINS IN SPINAL CORD TISSUE IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Mice overexpressing human G93A mutant SOD1 (mSOD) develop an ALS-like disease, and demonstrate robust abnormal hyperphosphorylation of proteins in the central nervous system (CNS). O-linked glycosylation by N-acetylglucosamine (O-GlcNAc) is a common form of protein post-translational modification that has been found to occur on many proteins at serine and threonine residues that are also known to be phosphorylated. Recently, it has been reported that phosphorylation of neurofilament is regulated by O-GlcNAc where O-GlcNAc and phosphorylation levels on neurofilament are reciprocal. O-GlcNAc modification of proteins likely plays an important role in nerve cell biology and neurodegenerative disease.

Objective: To evaluate protein-O-GlcNAc levels and describe the localization of O-GlcNAc-modified proteins in spinal cords of mSOD mice and wild-type littermates.

Methods: Tissue from mSOD and wildtype littermate mice were studied using an anti O-GlcNAc mouse monoclonal antibody where O-GlcNAc-modified protein levels in frozen spinal cord tissue were determined by Western blot. The regional and cellular localization of O-GlcNAc-modified proteins in spinal cord were identified by immunohistochemistry and fluorescent/confocal microscopy.

Results: O-GlcNAc-modified proteins were observed in Western blots of spinal cord tissue from mSOD and control mice. Multiple bands with a broad range of protein sizes were evident. Band densities of total protein levels for O-GlcNAc-modified proteins did not show a significant difference between mSOD and wild type mice, but some specific O-GlcNAc modified protein levels were different between mSOD mice and wild type littermates. Using densitometry analysis of immunoreactivity in spinal cord tissue, O-GlcNAc immunoreactivities were significantly decreased in ventral horn region in mSOD mice compared to controls. O-GlcNAc immunoreactivity was more extensive in grey matter than in white matter of the ventral horns of spinal cords. In grey matter, O-GlcNAc immunoreactivity was seen within some interneurons and some motor neurons in which the nucleus, perikaryon, and some dendrites, with especially intense reactivity in the perinuclear membrane of motor neurons. In both white and grey matter we observed many small, round cells that showed immunoreactivity against O-GlcNAc. Some of these cells were oligodendrocytes as confirmed with an antibody to CNPase.

Conclusion: To our knowledge, this is the first report to describe the distribution of O-GlcNAc immunoreactivity in spinal cords of mSOD mice. Further studies will be directed to identifying the proteins serving as substrates for O-linked glycosylation in the nervous system.

P158 DISTRIBUTION OF MUTATED VPS54 IN WOBBLER MOTOR NEURONS: RELATIONSHIP TO UBIQUITINATED INCLUSIONS

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Background: Ubiquitinated inclusions (UbIs) in motor neurons (MNs) of patients with ALS may contain aberrant proteins that would be harmful if not sequestered from the cell, including those due to genetic mutations, e.g., SOD1. Wobbler (*wr*) mouse MNs, which also contain UbIs, degenerate because of a mutation in *Vps54*, which encodes a protein member of the Golgi Associated Retrograde Protein (GARP) complex transporting endosomes to Golgi apparatus (GA). How mutant Vps54 protein results in neuronal degeneration and formation of UbIs is unknown but may be related to failed cargo transport and accumulation in UbIs.

Objectives: To develop a novel antibody against Vps54 for protein localization within motor neurons and to determine whether mutant protein is mislocalized to UbIs in *wr* motor neurons.

Methods: Rabbit polyclonal antibodies were developed against a 15-mer peptide sequence of Vps54, affinity purified, and tested for specificity. Cervical spinal cord (CSC) sections from *wr* and wildtype littermates were immunostained with Vps54 antibody, lysate from HeLa cells expressing full-length Vps54 protein and CSC tissue homogenate were immunoblotted. Double-labeling of *wr* CSC sections was also performed with Vps54 and GM130 or ubiquitin (Ub) antibodies to determine relationship of mutant protein with GA and UbIs, respectively.

Results: Vps54 immunostaining formed a reticular or lattice-like network in the cytoplasm of most CSC neurons, especially in wildtype mice. This appeared closely apposed to but not colocalized with GA. Immunoblotting of HeLa cell lysate and CSC homogenate revealed a prominent band at ~98kD, the predicted mass of full-length Vps54. Both tissue immunostaining and protein immunoblotting disappeared if Vps54 antibody was preabsorbed with the 15-mer peptide. Double-label immunofluorescence with Ub and size exclusion filter trapping studies localizing Vps54 to UbIs will be reported.

Discussion and conclusions: We have developed an antibody against Vps54 which decorates an intracytoplasmic network complex lining the GA in wildtype motor neurons, as may be expected for the GARP complex. However, the amount and distribution of Vps54 immunostaining in the *wr* appears different, suggesting a mistargeting of this protein to early degradation and/or accumulation in UbIs. Use of the Vps54 antibody will aid our understanding of how disruption in intracellular trafficking in the *wr* results in MN degeneration, as has been described in the human ALS-related mutations ALS2 and ALS8.

P159 CHARACTERIZATION OF PROTEIN AGGREGATES IN THE G93A- SOD1 MOUSE REVEALS A POSSIBLE LINK BETWEEN OXIDATIVE STRESS AND AGGREGATION PATHOGENETIC PATHWAYS

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Background: Mutations in the Cu, Zn superoxide dismutase (SOD1) gene cause a familial form of amyotrophic lateral sclerosis (ALS) through the acquisition of yet unknown toxic properties, such as the ability to catalyze an aberrant oxidative chemistry or the propensity to form protein aggregates. Proteinaceous inclusions rich in mutant SOD1 and ubiquitin have been found in tissues

from ALS patients and in mutant SOD1 animals, even before disease onset. However, very little is known about the aggregate protein constituents and therefore about the actual role and mechanism of aggregation in ALS pathogenesis. We have recently shown that mutant SOD1 progressively accumulates in a Triton X-100-insoluble fraction from the spinal cord of the G93A SOD1 mouse model of ALS, and that part of insoluble SOD1 is oligoubiquitinated and therefore not targeted to the proteasome.

Objectives: To investigate the impact and role of aggregation in disease pathogenesis we attempted a comprehensive characterization of the proteins and their post-translational modifications isolated from a Triton X-100-insoluble fraction of G93A SOD1 mouse spinal cords at different stages of the disease.

Methods: Experiments were carried out on samples from spinal cords of SOD1 G93A mice at a presymptomatic, symptomatic and late stage of the disease. Proteins were separated by fluorescence-based two-dimensional electrophoresis technology and identified by MALDI-TOF mass spectrometry. Validations of the results were made by Western blot and immunohistochemistry analysis with a selective number of proteins.

Results: We have completed the proteomic characterization of the insoluble proteins from spinal cords of G93A SOD1 mice. They are cytoskeletal proteins, mainly intermediate filaments, several mitochondrial proteins, chaperones, proteins of the endoplasmic reticulum, proteins involved in metabolic pathways and signaling. Now we are completing the characterization of the post-translational modifications, including nitration, carbonylation and phosphorylation. Interestingly, we could see that the majority of the aggregated proteins are oxidized.

Conclusions: We have performed the first comprehensive characterization of protein aggregates in a mouse model of ALS. Identification of post-translational modifications, carbonylation and nitration, in protein aggregates has suggested a possible link between oxidative stress and aggregation pathways in ALS pathogenesis.

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P160 $P2X_4$ PURINERGIC RECEPTOR IMMUNOSTAINING REVEALS NON-APOPTOTIC NEURONAL DEGENERATION IN MOTONEURONS AND OTHER NEURONAL TYPES IN RODENTS OVEREXPRESSING MUTATED SOD1

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The distribution of P2X family of ATP receptors was analyzed in a rat model for amyotrophic lateral sclerosis (ALS) expressing mutated human superoxide dismutase ($mSOD1^{G93A}$). We observed that strong $P2X_4$ immunoreactivity was selectively associated with degenerating motoneurons in spinal cord ventral horn. Degenerating $P2X_4$-positive motoneurons did not display apoptotic features such as chromatin condensation, positive TUNEL reaction or active caspase 3 immunostaining. In contrast, these neurons showed other signs of abnormality, such as loss of the neuronal marker NeuN and recruitment of microglial cells with neuronophagic activity. Similar changes were observed in affected motoneurons of the cerebral cortex and brainstem in $mSOD1^{G93A}$ both in rat and mice. Degenerating $P2X_4$ cranial motoneurons were observed in the facial, ambiguous and trigeminal but not in the oculomotor nuclei. In addition, the exquisite ability of $P2X_4$ antibody to detect neuronal degeneration allows the identification of neuronal damage in brain regions not usually considered as affected in transgenic animal models of ALS. These include: tyrosine hydroxylase-positive noradrenergic neurons in the locus coeruleus, serotonin containing neurons in the reticular formation and Purkinje cells of the cerebellar cortex. In all these locations, $P2X_4$-positive neurons recruited microglial cells with neuronophagic activity, in a similar way to that observed in motoneurons. By Western blot, we detected two protein bands in the expected range of $P2X_4$ molecular weight in crude membrane fraction extracts of spinal cord both in control and transgenic animals. In addition, a 30 Kda band was detected in the soluble fraction of transgenic $mSOD1^{G93A}$ rat spinal cord, which was observed in increasing amounts from P30 to the terminal stages of disease. At present, we do not know whether 30 Kda is a protein that is unrelated to $P2X_4$ but shares certain epitopes with it, or whether it is a product of $P2X_4$ cleavage resulting from altered processing of this protein in ALS motoneurons. However, immunostaining of all of the described protein bands as well as tissue immunocytochemistry was eliminated when the antibody was pre-incubated with the immunizing peptide. It is suggested that abnormal trafficking and proteolytic processing of $P2X_4$ receptor protein may be linked to neuronal degeneration in transgenic rodent ALS models.

P161 OVEREXPRESSION OF HUMAN WILD-TYPE SOD1 (HWTSOD1) ACCELERATES DISEASE IN A G85R TRANSGENIC MOUSE MODEL

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Background: Approximately 10% of ALS cases are familial (FALS), and ~25% of FALS cases are caused by mutations in Cu/Zn superoxide dismutase type 1

(SOD1). Sporadic and FALS cases share similar neuropathological features, suggesting that they both may share a common final pathway of cell death. SOD1 aggregation has been proposed as key to the pathogenesis of FALS. A recent study found that expression of hWTSOD1 greatly shortened disease incubation in several transgenic mouse models (G93A, A4V and a truncated mutant SOD1, L126Z) (1) in contrast to a previous investigation that reported no change in disease in the G85R transgenic mouse following expression of hWTSOD1. Because the electrophoretic mobility of L126Z is different from hWTSOD1, biochemical studies of the L126Z/hWTSOD1 double transgenic mouse were able to demonstrate heterodimers between the truncated L126Z and hWTSOD1.

Objectives: 1) To determine whether there are differences in the effect of hWTSOD1 overexpression in a G85R transgenic mouse compared to other mutant SOD1 transgenic mice. 2) To test whether heterodimers are formed between hWTSOD1 and G85R (which has a different electrophoretic mobility than hWTSOD1).

Methods: We generated a transgenic mouse that carried a human G85R SOD1 gene. G85R/hWTSOD1 double transgenic mice were generated by crosses between heterozygous G85R mice and hWTSOD1 mice. Mice were genotyped for the presence of G85R and hWTSOD1.

Results: The single G85R transgenic mouse that we generated developed disease in ~11 months (329 ± 18.4 days) and died ~2 weeks later (survival: 347.5 ± 21.2 days). In contrast, 5 mice that carried the hWTSOD1 and G85R transgenes (that have been studied so far) developed disease as early as 154 days (170 ± 18.1 days) with as short a lifespan as 164 days (survival: 197 ± 20.5 days). After the onset of weakness, both single G85R and double G85R/hWT transgenic mice showed a similar rapid progression and duration of disease. There was MN loss in paralyzed G85R/hWTSOD1 double transgenic mice, but no neuropathological changes in littermates sacrificed at the same age carrying either the G85R or hWTSOD1 single transgene.

Discussion and conclusions: Our findings indicate that overexpression of hWTSOD1 greatly accelerates MN degeneration and shortens disease incubation time as well as lifespan in G85R transgenic mice. This disease acceleration may be associated with conversion of hWTSOD1 in MNs of the spinal cord from a soluble form to insoluble aggregates; biochemical studies are in progress.

Reference

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P162 ELUCIDATION OF THE MISFOLDING PROCESS OF THE CU, ZN SUPEROXIDE DISMUTASE: IMPLICATIONS FOR TREATING FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: The largest subset of cases of familial amyotrophic lateral sclerosis (fALS) is known to be caused by mutations in the Cu, Zn superoxide dismutase (SOD1) (1). Surprisingly, many such mutations have no effect on the normal enzymatic activity of this protein. Rather, the disease phenotype seems to result from a gain of cytotoxic function – namely, an increase in the propensity of the protein to misfold and to aggregate (1). Unfortunately, the misfolding process is poorly understood, and consequently, it is not known whether the cytotoxic species is some intermediate in the misfolding process or the aggregated protein itself.

Objectives: This study aims to generate a complete model of the sequence of events that occur as SOD1 misfolds. Such a model would enumerate the intermediates of the misfolding process, a prerequisite for identification of the cytotoxic species. In addition, a full understanding of the misfolding process is needed for the development of drugs designed to prevent formation of cytotoxic SOD1 species in order to treat fALS.

Methods: A number of biochemical and biophysical techniques have been employed to monitor the guanidine hydrochloride denaturation of human erythrocyte SOD1 in real time. An assay using 4-(2-pyridylazo)resorcinol (PAR), an absorbance-based metal indicator, was employed to quantify the release rates of copper and zinc ligands. Metal release data were fitted to a number of release models. Rates of conformational change in the beta barrel region of the protein were measured based on time-resolved tryptophan fluorescence experiments. Ongoing work includes using size-exclusion chromatography to measure rates of dimer dissociation, and repetition of experiments using mutant SOD1 to determine how fALS mutations affect the misfolding process.

Results: Metal release data were best described by a two-step sequential release model in which zinc release necessarily precedes copper release. Tryptophan release data show that beta barrel conformational changes proceed according to a multi-step sequential mechanism. These changes occur on a much longer timescale than metal release. Preliminary results indicate that dimer dissociation is an even slower process.

Discussion and conclusions: The use of a variety of time-resolved biochemical and biophysical techniques is allowing the elucidation of the misfolding process of SOD1. This process seems to involve the release of metal ligands as an early step, with zinc release preceding copper release. Additional conformational changes and dimer dissociation follow. It will be interesting to see which steps

in this sequence are disrupted by ALS mutations. Ultimately, this work will be useful to allow identification of the cytotoxic SOD1 species and to enable the development of drugs to block SOD1 cytotoxicity in order to treat fALS.

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P163 INVESTIGATION OF CU/ZN SUPEROXIDE DISMUTASE MISFOLDING AND AGGREGATION IN ALS USING CONFORMATION-SPECIFIC ANTIBODIES

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Background: Mutations in Cu/Zn superoxide dismutase (SOD1) are the cause of 2–5% of ALS cases. The propensity of such mutants to misfold and/or aggregate is believed to be a key aspect of their toxicity to motor neurons. Based on earlier data suggesting that monomeric SOD1 is an intermediate in the unfolding/aggregation pathway of SOD1, we have developed an antibody (the SEDI antibody: SOD1 Exposed Dimer Interface) which recognizes monomeric/misfolded, but not folded, SOD1. This antibody allowed the first *in vivo* detection of monomeric/misfolded SOD1 in mouse models of ALS, as well as in human tissue (1).

Objectives: To study the tissue distribution of monomeric/misfolded SOD1 in the G93A mouse model of ALS, and to develop immunological methods to study SOD1 misfolding/aggregation *in vitro* and *in vivo*.

Methods: Using the SEDI antibody, we developed a sensitive and specific competition enzyme-linked immunosorbent assay (competition ELISA) for monomeric/misfolded SOD1. This ELISA was used to detect monomeric/misfolded SOD1 in tissue extracts from the G93A mouse model of ALS. The tissues studied included brain, spinal cord, liver, heart, muscle, kidney and spleen.

Results: We found that monomeric/misfolded SOD1 only accumulates to high levels in brain and spinal cord, strongly suggesting that such conformational species are involved in disease mechanisms. We also present data showing that the SEDI antibody can be used successfully to study the folding/unfolding mechanism of SOD1 *in vitro* using an ELISA format. Finally, we describe the development of ELISA-based assays for SOD1 pharmacological chaperones.

Discussion and conclusions: The ELISA developed in this work will be useful in studying SOD1 misfolding in tissues from human ALS patients, potentially establishing monomeric/misfolded SOD1 as a useful biomarker for this disease. This work highlights the power of conformation-specific antibodies to unravel the biochemical mechanisms underlying protein misfolding diseases.

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P164 IDENTIFICATION OF CELLULAR FACTORS INTERACTING WITH MUTANT SOD1

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Background: Different murine transgenic ALS models express similar mRNA levels in the spinal cord, but the amounts of human SOD1 protein in the spinal cord differ widely, from half (G127X, C-terminal truncation), equal (G85R) to 17-fold (G93AGur) and 20-fold (D90A) higher than endogenous murine SOD1 levels. The G127X and G85R SOD1s in spinal cords are disulfide-reduced, inactive, likely to misfold and enriched relative to other organs, suggesting inefficient degradation of such SOD1 forms. Mis/unfolding of proteins leads to exposure of the hydrophobic core and increased binding in hydrophobic interaction chromatography (HIC). 100% of the G127X and 80% of G85R in the spinal cords bind to HIC columns. In the more stable SOD1 models, G93A and D90A, subfractions of the SOD1 in spinal cord extracts bound to the HIC columns, 3% and 1% respectively. Much less of the SOD1 in brain and peripheral organs bound in the HIC. The hydrophobic soluble SOD1 which is long-term enriched in spinal cords could be responsible for the cytotoxicity.

Objectives: Misfolded SOD1 exposing sticky hydrophobic structures are likely to interact with cellular factors. In this study we try to find factors that interact with different forms of human SOD1. Such interactions might induce the cytotoxic effects of mutant SOD1s.

Methods: Recombinant human wt and mutant SOD1s are coupled to CNBr-activated Sepharose gels. After coupling, the prosthetic Zn- and Cu ions can be removed and the C57-C146 disulphide bond can be removed for studies of holo/apo and disulphide oxidised/reduced proteins. The gels with coupled SOD1 proteins are then incubated with rat spinal cord homogenates. After rigorous washing to remove unspecific binding to the sepharose gel, the interactions are broken in high denaturing conditions (4M Gua-HCl) and the interacting factors are collected. To identify these factors, 2D-gel

electrophoresis and MALDI-TOF mass spectrometry are used.

Results: Proteins previously shown to bind to (mutant) SOD1s such as CCS and HSC71 have been identified showing the validity of the protocol. We are now focusing on novel SOD1 interactions that could be involved in the pathogenesis of ALS.

Discussion: Identification of these essential cellular factors interacting with misfolded SOD1 might provide new ideas for the elucidation of the pathogenic mechanism behind ALS that involves misfolded SOD1.

P165 MUTANT-SOD1-INTERACTING PROTEINS: A PROTEOMIC ANALYSIS IN SOD1^{LEU126DELTT} TRANSGENIC MICE

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Background: A wide range of cellular functions are involved in ALS motor neurons, including excessive excitatory toxicity, protein misfolding (aggregation), abnormal calcium metabolism, altered axonal transport, and so on. In ALS with SOD1 mutation, the altered SOD1 molecule is wholly responsible for these consequences. The precise mechanisms involved, however, have yet to be fully understood.

Objectives: To identify mutant-SOD1-interacting proteins in the spinal cord of SOD1^{Leu126delTT} transgenic mice with FLAG-tag sequence at C terminal of the SOD1 (DF7 TgM) using mass spectrometry based proteomics. This may provide new insights into disease pathogenesis.

Methods: Whole spinal cord was obtained from DF7 TgM before and after the onset of disease. As controls we used WF2 TgM that expressed wild-type SOD1 with FLAG sequence at the C terminal. FLAG-tagged SOD1 and cross-linking proteins were enriched using ant-FLAG M2 affinity gel. The proteins were digested and then subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Results: We identified more than 20 proteins that cross-reacted with mutant SOD1 in DF7 TgM. One of them was HSC70 (HSPA8), which in a previous report had been found to bind to mutant SOD1. We were unable to identify mouse intrinsic SOD1 protein as a mutant-SOD1-interacting protein. In WF2 TgM, on the other hand, only 4 proteins interacted with SOD1, one of which was mouse SOD1.

Discussion and conclusions: Our proteomic data revealed comprehensive as well as specific protein-protein interactions in the spinal cord of DF7 TgM. As previously predicted, SOD1^{Leu126delTT} did not interact with mouse SOD1. This fact strongly suggests that SOD1^{Leu126delTT} protein exists as monomer and may cause inadequate protein formation in such cases as HSC70. Mass spectrometry based proteomics can be a powerful tool for identifying mutant-SOD1-interacting proteins and helps elucidate the disease mechanisms of FALS.

P166 STUDY OF IONIC CONDUCTANCES OF MITOCHONDRIA IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Recent findings have shown that mutSOD1 proteins bind and aggregate with Bcl-2 in spinal cord mitochondria of amyotrophic lateral sclerosis (ALS) mice and patients. Given the protective role of the anti-apoptotic members of the Bcl-2 family of proteins in preventing the disruption of mitochondrial integrity, specifically by regulating the outer mitochondrial membrane (OMM) permeability, it is reasonable to hypothesize that mutSOD1 exerts its toxic properties by indirectly (through binding with Bcl-2) or directly affecting the conductances in the OMM. Dysregulation of OMM conductances would ultimately lead to swelling of mitochondria, release of apoptogenic factors like cytochrome c, activation of the caspase-mediated apoptotic cascade and cell death.

The overarching goal of this work is to provide evidence on whether ion channels of the OMM function abnormally in ALS.

The current knowledge of the biophysical properties of the OMM refers to artificial systems and, to our knowledge, a detailed characterization of the OMM conductances in acutely isolated mammalian mitochondria has never been reported.

In this study we applied the patch-clamp technique to the OMM of acutely isolated mitochondria from the spinal cord of naïve control mice and G93A-SOD1 ALS mice.

Recordings in symmetrical conditions (150 mM KCl) of spinal cord mitochondria isolated from control mice revealed the presence of a background conductance of around 100 pS always open at any given potential between −160 and +160 mV which showed to be slightly cationic selective. Interestingly, upon addition of MgATP in the pipette a large voltage-dependent conductance of up to 2.5 nS appeared only at voltages between −30 and +10 mV. Estimates of the pore diameter based on molecule exclusion and on conductance measure suggest that the MgATP-modulated channel is permeable to molecules up to 3000 MW. Preliminary recordings from late pre-symptomatic (80 day old) and symptomatic (130 day old) G93A mice showed that in addition to the conductances observed in control mice, mitochondria from G93A mice possess a large multiconductance channel open at all voltages.

The large multi-conductance activity detected in G93A-SOD1 ALS mice might be indicative of the pathological

effects of mutSOD1 on the OMM of spinal cord mitochondria.
Understanding how the OMM conductances are altered in ALS and identifying the specific channels that are targeted by mutSOD1 has potential implications for the identification of drugs that specifically restore channel function.

P167 DEFINING THE NATURE OF MITOCHONDRIAL ASSOCIATION OF ALS-LINKED SOD1 MUTANTS

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Dominant mutations in the ubiquitously expressed copper/zinc superoxide dismutase (SOD1) are causative for a subset of inherited amyotrophic lateral sclerosis (ALS). More than 116 mutations scattered through the SOD1 protein (itself only 153 amino acids) have been identified in ALS patients. Despite the inherent variability in biochemical properties conferred by these mutations, all mutant proteins studied have been reported to be selectively associated with mitochondria in affected spinal cords, but not in unaffected tissues. In the current effort, we extend this observation to report that: 1) A proportion of mutant G85R, G127X, G93A SOD1 are mitochondrial bound (as demonstrated by their flotation to the same density as mitochondria); 2) Low electrophoretic mobility SOD1 adducts are mitochondrially bound; 3) Mitochondrially associated, dismutase inactive mutants (H46R, G85R) are bound to the cytoplasmic mitochondrial face, as demonstrated by protease sensitivity; 4) Half of mitochondrially associated, dismutase active SOD1 mutant (G93A) is tightly associated (alkali resistant) with spinal cord-derived mitochondrial membranes, none is so tightly associated with cortical mitochondria; 5) All mitochondrially associated dismutase inactive mutants (H46R and G85R) are tightly bound to spinal cord mitochondrial membranes, but none is so tightly associated with cortical mitochondria; 6) Dismutase inactive, mitochondrially associated mutant G85R is not a soluble matrix protein (digitonin treatment of mitochondria releases almost all soluble matrix proteins but no G85R); and 7) A proportion of wild type or wild type-like, dismutase active mutants are trafficked into a soluble mitochondrial compartment, but a proportion of misfolded, inactive mutants appear trapped in transit across a mitochondrial membrane.
Thus, mitochondrial association of mutant SOD1 preferentially within tissues at risk in ALS is common to SOD1 mutants of varied biochemical character.

P168 PROGRESSIVE MOTOR NEURONOPATHY (PMN): A CRITICAL ROLE OF THE TUBULIN CHAPERONE TBCE IN AXONAL TUBULIN ROUTING FROM THE GOLGI APPARATUS

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Background: Axonal degeneration represents one of the earliest pathological features in motor neuron diseases linked to mutations in SOD1, Alsin or SMN.

Objectives: We studied the molecular and cellular mechanisms of axonal degeneration in progressive motor neuronopathy *(pmn)* mice (1) mutated in the tubulin-specific chaperone TBCE (2,3).

Results: We demonstrate that TBCE is a peripheral membrane-associated protein that accumulates at the cis-Golgi apparatus. In *pmn* mice, TBCE is destabilized and lost from the Golgi apparatus of motor neurons and microtubules are rarefied in distal axons. The axonal microtubule loss proceeds retrogradely in parallel with the axonal dying back process. These degenerative changes are inhibited in a dose-dependent manner by transgenic TBCE complementation which restores TBCE expression at the Golgi apparatus. In cultured motor neurons, the *pmn* mutation, RNAi-mediated TBCE depletion and Brefeldin A-mediated Golgi disruption all compromise axonal tubulin routing.

Conclusion: We conclude that motor axons critically depend on axonal tubulin routing from the Golgi apparatus, a process that involves TBCE and possibly other tubulin chaperones.

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P169 SELECTIVE GENE ABLATION OF MUTANT SOD1 IN ASTROCYTES SIGNIFICANTLY SLOWS DISEASE PROGRESSION OF ALS MICE

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Background: Dominant mutations in the ubiquitously expressed Cu/Zn superoxide dismutase (SOD1) lead to amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting adult motor neurons. Although ubiquitous expression of mutant SOD1 provokes progressive, selective motor neuron degeneration in human and rodents due to an acquired toxic property(ies) of the mutant, the cell types that contribute to the onset and progression of the motor neuron disease are of interest to design therapies. In this regard, we have previously shown that onset and progression were determined by motor neurons and microglia, respectively, however, the contribution of other cell types is not known.

Objectives: 1) To test non-cell autonomous motor neuron death in ALS mice. 2) To determine the cell types in which mutant SOD1 acts to generate toxicity to the motor neurons in ALS.

Methods: We have generated novel chimeric mice in which all motor neurons express mutant SOD1. This was achieved by making mouse chimera of $SOD1^{G37R}$ and Olig1/2-deficient mice ($Olig^{-/-}$), which cannot generate motor neurons and oligodendrocytes. In addition, to delete mutant SOD1 within astrocytes or postnatal motor neurons, $LoxSOD1^{G37R}$ mice, which ubiquitously express deletable mutant SOD1 by the action of Cre recombinase, were mated with GFAP-Cre-LacZ or VAChT-Cre mice, respectively. The disease onset, duration, and survival of these cohorts were monitored.

Results: $Olig^{-/-}$::$SOD1^{G37R}$ chimeras escaped motor neuron disease for at least 40 days after all germline $SOD1^{G37R}$ mice had died. The majority of mutant-expressing motor neurons in chimeric mice escaped degeneration and exhibited only minor microglial and astrocytic activation. Using $LoxSOD1^{G37R}$ mice, removing mutant SOD1 from motor neurons delays the age of disease onset, confirming mutant action in neurons as an initiating factor in triggering disease. More importantly, silencing of SOD1 mutant expression selectively within astrocytes has minimal effect on age of disease onset, but significantly slows disease progression (extending survival after onset by 56 days).

Discussion and conclusions: These two experiments provided conclusive evidence supporting non-cell autonomous motor neuron death in ALS mice. The timing of disease progression is determined by astrocytes and microglia, which are targets for the therapy to slow disease progression in ALS.

P170 MAPPING GENETIC MODIFIERS IN SOD1-G93A CONGENIC MOUSE STRAINS

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Background: Mutations in the gene encoding superoxide dismutase 1 (SOD1) cause amyotrophic lateral sclerosis (ALS) in humans. A large number of investigations have used transgenic mice over expressing a human mutant form of SOD1 that has a glycine replaced by an alanine at position 93 (hSOD1-G93A). The transgenic mice used in the majority of these studies were maintained on a segregating genetic background (C57BL/6J × SJL/J) which results in no two animals being genetically identical.

Objective: Our studies sought to better understand the influence of genetic background and to search for potential genetic modifiers of SOD1-induced amyotrophic lateral sclerosis (ALS). To this end we bred the hSOD1-G93A transgene onto several inbred mouse strains to create full congenic lines on C57BL/6J, ALR/LtJ, DBA/2J, BALB/cByJ and NOD.Cg-*Rag1*−/−.

Methods: Mice were monitored for the appearance of overt symptoms after each backcross generation. Several of the inbred strain backgrounds caused the timing of the appearance of overt symptoms to shift and from these we selected two strains, C57BL/6J and ALR/LtJ, which differed widely in disease onset for further analysis. Once the strains were fully inbred preliminary phenotypic characterization was conducted including growth curves, axon counts in ventral roots and peripheral nerves as well as innervation status at the neuromuscular junction. Matings were also set up to produce N2 backcross mice for QTL analysis using single nucleotide polymorphisms (SNPs).

Results: C57BL/6J and ALR/LtJ differed significantly in their 50% survival time (159.4 ± 0.9 days vs. 110.8 ± 1.4 days respectively). The absolute number of days from onset to death was also significantly less in ALR/LtJ mice (22 ± 2.4 vs, 15.2 ± 2.4 days, respectively) but when expressed relative to total lifespan the strains were similar. Preliminary data from 176 ALR × (ALR × B6-SOD1(G93A)) N2 mice from a mapping cross between these two strains has identified a major B6-dominant modifier located on Chr. 17 and 3 additional suggestive loci on chromosomes 4, 11 and 19. Additional samples are currently being analyzed and more animals from a backcross in the opposite orientation (B6 × (B6 × ALR-SOD1(G93A)) are being generated to confirm and narrow the identified loci. Phenotypic characterization has revealed no obvious qualitative differences in the presentation of the disease. Transgenic mice of both strains show the expected reductions in body weight, ventral root and peripheral axon counts and loss of neuromuscular innervation. Preliminary analysis suggests that the extent of degeneration was similar between strains when expressed relative to disease onset.

Conclusions: Additional SNP analysis is required to narrow the modifier loci and identify potential candidate

genes. Together, phenotypic results suggest that the differences in genetic background between these strains do not modify the nature of ALS disease caused by the SOD1-G93A mutation but rather significantly alter the latency of the presymptomatic period.

P171 MUTATED VALOSIN-CONTAINING PROTEIN ACCELERATES ONSET AND PROGRESSION OF DISEASE IN SOD1 ^{G93A} TRANSGENIC MICE

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Background: The SOD1^{G93A} transgenic mouse is an established animal model of human familial ALS. 20–25% of familial ALS is caused by SOD1 mutations with an unknown gain of toxicity. A subgroup of ALS is associated with frontotemporal dementia (FTD). FTD with autosomal dominant inclusion body myopathy associated with Paget disease of bone (IBMPFD) is caused by mutation of the Valosin-Containing Protein (VCP) gene. VCP performs a variety of essential cellular functions including Golgi assembly, apoptosis and ubiquitin proteasome and endoplasmic reticulum-associated protein degradation. Suggested mechanisms for SOD1 toxicity include apoptosis, protein misfolding and aggregation. It is unknown if VCP mutation influences SOD1 toxicity.

Objectives: To explore the effect of mutant VCP on the onset and progression of SOD1^{G93A} transgenic mice.

Methods: SOD1^{G93A} transgenic mice were previously established in our laboratory. The R155P mutation of VCP was identified in a IBMPFD family and introduced by site-directed mutagenesis to engineer the VCP^{R155P} transgene. A 17.9kb human genomic DNA fragment containing the entire VCP gene was used for microinjection and the VCP transgenic mice were crossbred with SOD1^{G93A} to generate SOD1^{G93A}/VCP^{R155P} double transgenic mice. Transgenes were identified by PCR, DNA sequencing and Southern blot analysis. Mice were weekly observed and evaluated for weight again and Rotarod analysis. Rotarod was performed three times for each session. VCP immunohistochemistry was performed with polyclonal anti-VCP antibody using the ABC system.

Results: We generated four SOD1^{G93A}/VCP^{R155P} double transgenic mice, two siblings of VCP^{R155P}, two siblings of SOD1^{G93A} and two nontransgenic siblings. SOD1^{G93A}/VCP^{R155P} double transgenic mice stopped gaining weight from 61 day onwards and the onset of hind limb paralysis was at 77.7 ± 3.9 days and end-stage was at 84.3 ± 4.6 days. The SOD1^{G93A} and VCP^{R155P} mice were not affected at the time of paralysis of the double transgenic mice. The average onset of paralysis in SOD1^{G93A} mice ($n=273$) was 120.4 ± 8.2 days and end-stage time of SOD1^{G93A} mice ($n=397$) was 128.5 ± 10.7 days. The SOD1^{G93A}/VCP^{R155P} vastus lateralis muscle showed remarkable muscle fiber atrophy and fiber size variation, but without obvious rimmed vacuoles. Muscle morphology of SOD1^{G93A} and VCP^{R155P} mice of the double cross siblings were normal at the time SOD1^{G93A}/VCP^{R155P} reached end stage. VCP immunohistochemistry analysis and characterization of the muscles, brain and spinal cord is currently underway.

Discussion and conclusions: VCP mutation significantly accelerates the onset and progression of disease in SOD1^{G93A} mice. SOD1^{G93A}/VCP^{R155P} double transgenic model provides new avenues to study the interactions of cellular processes in ALS and FTD and might provide insight into the pathogenesis of G93A ALS mice.

P172 ANALYSIS OF THE ROLE OF CYTOPLASMIC DYNEIN IN THE TOXICITY OF SOD1^{G93A}

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Background: SOD1^{G93A} transgenic mice develop an ALS-like phenotype. Previously, we linked a missense point mutation in the gene encoding the heavy chain subunit of cytoplasmic dynein to motor neuron degeneration in the Legs at odd angles (*Loa*) mouse (1). Recently, we showed that the *Loa* mutation delays disease onset and increases the life span of double mutant (*Loa*/SOD1^{G93A}) transgenic mice when compared with their SOD1^{G93A} littermates. Moreover, in contrast to their SOD1^{G93A} littermates at late stages of the disease, the *Loa*/SOD1^{G93A} mice retained their normal muscle function with no significant loss of motor neurons (2).

Objectives: To elucidate at the molecular level the role of the *Loa* mutation in attenuating the phenotype of SOD1^{G93A} transgenic mice.

Methods: We have crossed the SOD1^{G93A} with *Loa* mice and the *Loa*/SOD1^{G93A} mice from this cross are being analyzed and compared with their SOD1^{G93A}, Loa/+ and +/+ littermates. Approaches such as *in vivo* and *in vitro* protein-protein interaction assays, cell fractionation and global proteomics are being exploited on brains and spinal cords of *Loa*/SOD1^{G93A} and their littermates at pre- as well as post-natal stages to investigate the possibility of interactions between SOD1^{G93A} and the dynein-dynactin complex during disease development.

Results: Our previous *in vitro* protein-protein interaction data did not conclude that there is a direct interaction between members of dynein-dynactin complex from mouse brains and the bacterially expressed wild-type SOD1 and SOD1$^{A4V,\ G37R,\ G85R}$ or ^{G93A}. Using a

polyclonal anti-SOD1 antibody and brain tissue extracts from $SOD1^{G93A}$ transgenic mice at their end-stage of the disease, we have co-immunoprecipitated the dynein-dynactin complex along with $SOD1^{G93A}$ protein. On the other hand, antibodies to dynactin p-150 or dynein intermediate chains could not pull-down the $SOD1^{G93A}$ protein from the same brain samples. Furthermore, the same anti-SOD1 antibody failed to co-immunoprecipitate the dynein-dynactin complex from brain tissues isolated from E16 embryos of the $SOD1^{G93A}$ or their *Loa*/$SOD1^{G93A}$ littermates.

Discussion: Our results suggest that association between $SOD1^{G93A}$ and the dynein-dynactin complex may not be due to a direct interaction but rather due to trapping of dynein/dynactin proteins within $SOD1^{G93A}$ aggregates that are formed during disease progression. Analysis of other disease stages is now underway.

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P173 THE NEUROPROTECTIVE EFFECTS OF DIMEBON IN THE G93A SOD1 MOUSE MODEL OF ALS

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Background: Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's Disease) is the most common adult onset motor neuron disorder in humans. Worldwide prevalence of ALS is estimated to be 0.4 to 2.4 cases per 100,000 persons. In the United States, the prevalence is 15,000 to 30,000 cases and an estimated 5,000 new cases are diagnosed each year (1). The condition has no known cure, but several drugs have been found to delay symptom development and or death in mutant SOD1 mice, an animal model for ALS (2). Dimebon is orally active small molecule which has been shown to have general neuroprotective effects as well as enhancing cognition in animals. A small pilot trial in Alzheimer's patients treated with Dimebon reported beneficial effects (3). These studies suggest that Dimebon might be a candidate for treating other neurodegenerative diseases.

Objectives: In the present study, we have evaluated the effects of dimebon on ALS, using an animal model of this disease, the G93A/SOD1 transgenic mouse. These animals have a mutant form of superoxide dismutase, which is associated with an inherited form of ALS. Over time these mice develop neurodegenerative disease with progressive loss of motor function similar to the symptoms of ALS.

Methods: We administered dimebon in the drinking water of SOD1 mice at daily doses of 3, 10 or 30 mg/kg. Treatment began when the animals began showing motor function deficits (limping) typical of the Stage III of the disease. The time for treated and untreated control animals to reach Stage II (paralysis in one or more limbs) and Stage I (negative for reflexes, unable to right itself when placed on its back) was determined.

Results: Statistical analysis was performed for male or female animals in each dose group as well for all treated animals taken as a single group. There were no significant differences between controls and animals receiving daily dimebon in the time to reach stage 2.

Acknowledgement: This study was supported in part by Medivation Inc. and Forbes Norris ALS/MDA clinic of California Pacific Medical Center Research Institute

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P174 EVIDENCE FOR THE NEUROPROTECTIVE EFFECT OF ANGIOGENIN IN MOTONEURONS BOTH *IN-VITRO* AND *IN-VIVO*

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Background: A recent investigation of hypoxia-related genes in ALS patients has identified novel disease-specific mutations in angiogenin (ANG) in both familial and 'sporadic' ALS patients (1). ANG has never previously been associated with ALS, and therefore its potential role in the pathogenesis of ALS is largely undetermined.

Objectives: To examine the potential neuroprotective effect of angiogenin in both *in-vitro* and *in-vivo* models of motoneuron degeneration.

Methods: *In-vitro* primary motoneuron cultures were exposed to the excitotoxin AMPA (50μM, 24hrs), and the effect of co-treatment with Ang on cell viability and motoneuron survival was determined. *In-vivo* we examined the effect of Ang protein treatment in $SOD1^{G93A}$ mice by treating mice daily with Ang and monitoring disease onset and progression using stride length analysis, as well as recording body weight, paw grip endurance test performance, motoneuron survival and lifespan.

Results: Here we report that ANG has significant neuroprotective effects both *in-vitro* and *in-vivo*. *In-vitro*, treatment with Ang (100ng/ml) protected motoneurons from excitotoxic cell death, such that 74%($\pm$4.5) motoneurons survived in Ang co-treated cultures compared to

only 48%(±4.1) motoneurons in AMPA-treated cultures ($p < 0.05$). *In-vivo*, treatment with Ang protein (1ug/daily) significantly delayed disease onset and progression, and increased motoneuron survival at later stages of the disease such that at 90 days, 498(±13.7) motoneurons survived in Ang-treated $SOD1^{G93A}$ mice compared to only 405(±12.8) in untreated $SOD1^{G93A}$ littermates. This resulted in a significant increase in lifespan in treated $SOD1^{G93A}$ mice.

Conclusion: Our study suggests that ANG delivery may be a novel approach for the treatment of ALS.

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P175 HUMAN MESENCHYMAL STEM CELLS AMELIORATE THE PHENOTYPE OF SOD1-G93A MICE

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, neurodegenerative disease, currently without any effective therapy. Multiple advantages make mesenchymal stem cells (MSCs) a good candidate for cellular therapy in many intractable diseases such as stroke and brain injury. Until now, no irrefutable evidence exists regarding the outcome of MSCs transplantation in the mouse model of ALS.

Objectives: The present study was designed to investigate the therapeutic potential of human MSCs (hMSCs) in the mouse model of ALS (SOD1-G93A mice).

Methods: hMSCs were isolated from iliac crest aspirates from healthy donors and kept in cell cultures. hMSCs of the 5th passage were delivered intravenously into irradiated presymptomatic SOD1-G93A mice. Therapeutic effects were analyzed with survival analysis, rotarod test, motor neuron count in spinal cord and electrophysiology. The engraftment and *in vivo* differentiation of hMSCs were examined in the brain and spinal cord of hMSCs transplanted mice.

Results: After intravenous injection into irradiated presymptomatic SOD1-G93A mice, hMSCs survived more than 20 weeks in recipient mice, migrated into parenchyma of brain and spinal cord and showed neuro-glia differentiation. Moreover, hMSCs transplanted mice showed a significantly delayed disease onset (14 days), an increased life span (18 days) and a delayed disease progression compared to untreated mice.

Conclusion: Our data document the positive effects of hMSCs transplantation in the mouse model of ALS. It may signify the potential use of hMSCs in treatment of ALS.

P176 THE INFLUENCE OF CENTRAL AND PERIPHERAL TRAUMA ON THE ONSET OF DISEASE AND SURVIVAL IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: It is not clear from clinical epidemiological studies whether trauma has a negative impact on the onset and progression of ALS. Most such studies have inherent pitfalls due to the wide spectrum of possible injuries and clinical pictures.

Objectives: To analyze whether head, spinal or limb trauma will affect the onset of the disease and/or progression of the disease in SOD1 transgenic mice.

Methods: 100 mice obtained from breeding G93A SOD1 transgenic males and wild-type females were randomly assigned to four equal groups: mild head trauma, mild trauma to the neck, to one hindlimb and a control group. Each group was expected to include approximately 1:1 transgenic and wild-type mice. Standardized trauma was performed in all mice at age 6 weeks. PCR to identify transgenic mice was performed after death or at age 5 months, to avoid further trauma caused by cutting the tail ends. All mice were evaluated one day before trauma and every week thereafter by clinical grading of motor weakness, rotarod, inclined plane test, limb extension reflex, horizontal bar and staircase test, survival.

Results: There were 51 transgenic mice in the present study group, distributed as follows: 12 mice in each group of trauma, 15 in the control group. There was no statistically significant difference in any of the measured variables between the groups of injured mice and the controls. Mild trauma, to all three different locations, did not change disease onset, progression rate or survival.

Conclusion: Our results do not support the concept of a negative influence of trauma in ALS.

P177 EXPOSURE TO 50 HZ MAGNETIC FIELD AND ALS: ARE THEY ASSOCIATED?

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Background: Environmental insults may be factors predisposing to or triggering amyotrophic lateral sclerosis. But, to date, none has been identified. Some epidemiological studies have suggested that occupational

exposure to electromagnetic field (EMF) is associated with ALS (1–5).

Objectives: To evaluate for the first time, whether exposure of SOD-1 mice to 50 Hz magnetic fields (MF) could activate ALS processes.

Methods: SOD-1 mice were purchased from Jackson Laboratories (USA). Eight mice per group were exposed to 50 Hz MF at two levels (100 and 1000 μT). Exposures began before the onset of ALS clinical signs at 10 weeks. Exposure lasted 2 hours/day, 5 days/week for 7 weeks. Sham-exposed mice (placed in inactive coils) were included in this protocol. Body weight, survival, and motor impairment using the Rotarod test (15 rpm, 180 s) were monitored. Mice were sacrificed when they were unable to roll over within 30 sec after being pushed to their side, and this time point was recorded as the time of death. Mice were perfused transcardially with PBS-heparin, followed by 4% paraformal-dehyde in PBS. The brain and the spinal cord were dissected, cryoprotected in 20% sucrose and kept at -80°C until immunolabelling. Immunohistochemistry analyses of brain and spinal cord sections are being conducted to detect radical stress markers.

Results: In the exposed groups, no significant differences were measured, as compared to the sham-exposed animals, on the following parameters: weight, Rotarod test and survival.

Discussion and conclusions: 50Hz magnetic fields at two exposure levels do not seem to modify ALS progression. Available experimental data do thus not provide evidence for any link between occupational exposure to EMF and ALS.
Our data complement those from epidemiological studies related to risk assessment of neurodegenerative diseases in populations occupationally exposed to MF.

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P178 FIRST LINE SCREEN OF NEUROMUSCULAR SYNAPTIC PHENOTYPES IN ETHYLNITROSOUREA (ENU)–MUTAGENISED MICE USING CONFOCAL MICROENDOSCOPY

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Background: Severing the motor nerve supply to skeletal muscle normally triggers rapid Wallerian degeneration (WD). In homozygous *Wld*^{S} mutant mice, axon degeneration is delayed by expression of an *Nmnat/Ube4b* chimeric gene: disconnected axons are preserved for up to three weeks. However, in heterozygous *Wld*^{S} mice axotomy-induced degeneration of presynaptic motor nerve terminals occurs at a normal rate (1–2 days). This observation supports a compartmental model of neurodegeneration, according to which motor neuron soma, axon and nerve terminals (NMJ) degenerate in response to trauma or disease by different sub-cellular mechanisms. Discovery of gene mutations that selectively protect NMJ from WD would validate this hypothesis.

Objective: We designed a high-throughput screen of mice mutagenised by ethylnitrosourea (ENU), with the objective of discovering covert neuromuscular phenotypes following axotomy *in vivo*.

Methods: Our phenotypic assay uses a 1500 μm fibre-optic probe connected to a Cellvizio confocal microendoscope (Mauna Kea Technologies). The procedure is minimally invasive yet can resolve intact and degenerating axons and synapses in living anaesthetised transgenic mice, using expression of yellow fluorescent protein (YFP) in motor neurones as a biomarker. We use *Wld*^{S} mice as a sensitized background, examining for either additive synaptic protection or block of axonal protection following axotomy in the F1 offspring of ENU $\times$ *YFP-Wld*^{S} crossbred mice.

Results: None of the 38 ENU lines studied thus far has shown evidence of interaction with the *Wld*^{S}/+ phenotype. In addition, we tested the ability of the confocal microendoscope to detect phenotypic features of the ENU neuromuscular mutant *ostes*. Synaptic abnormalities were revealed by the Cellvizio in these mutant mice.

Conclusion: The data provide proof-of-principle that confocal microendoscopy is an effective tool for screening of neuromuscular phenotypes.

P179 GENERATION OF A NEW MODEL-SYSTEM TO STUDY AMYOTROPHIC LATERAL SCLEROSIS DISEASE BY NON INVASIVE IMAGING

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Background: Amyotrophic lateral sclerosis (ALS) is a late onset neurological disease characterized by progressive spinal motor neuron degeneration associated with paralysis and eventually death. Transgenic mice expressing a mutant superoxide dismutase 1 (SOD1) develop phenotype with many pathological features resembling human familial and sporadic ALS. Although major clinical symptoms in ALS arise from neurodegeneration and death of motoneurons, recent studies suggest that non neuronal cells could play a role in the toxicity to motor neurons. However, their precise role in onset and progression of the disease remain unknown.

Objectives: To further investigate the role of non-neuronal cells in the disease onset and progression through development of a mouse model for live imaging of astrogliosis in ALS.

Methods: As a glial fibrilary acidic protein (GFAP) is strongly up-regulated in ALS, we used it as a hallmark of astrogliosis to create our model. We thus crossed mice carrying the firefly luciferase gene under the transcriptional control of mouse GFAP promoter (GFAP-luc, Xenogen, CA) with mice carrying the $SOD1^{G93A}$ mutation. The double transgenic GFAP-luc/$SOD1^{G93A}$ mice were used in the study as well as GFAP (wt)-$SOD1^{G93A}$ and GFAP-luc as controls. Live imaging was performed weekly starting from postnatal weeks 3–4 till the end stage of the disease. Loss of extension reflex, weight loss and motor deficits were used as indicators of clinical symptoms.

Results: The results were obtained from 19 female/male mice matched-age littermates and were compared to adequate controls (n=7). Data collected by *in vivo* imaging showed that photon emission/GFAP signal was first detected at the lumbar spinal cord area. The signal first arose from small multiple areas of astrocyte activation which then converged into a larger signal around 80–100 days of age. The correlation analysis between live imaging and behaviour data revealed that increase in GFAP signal in the spinal cord at 70–80 days correlated with the initial disease onset (loss of extension reflex). Moreover the peak signals arising from the spinal cord at approx. 100 days correlated with the abrupt onset of sensorimotor deficit and paralysis. The end-stage of the disease (approx 133d) was characterized by an increase of bioluminescent signals arising from the different brain structures that coincided with the loss of body weight.

Conclusion: GFAP-luc//$SOD1^{G93A}$ mice will provide a unique model for understanding disease pathology and longitudinal responses to drug testing.

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P180 AGE-DEPENDENT CHANGES OF CNS METABOLITES IN THE SOD1 MOUSE MODEL OF ALS

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Background: So far, *in vivo* magnetic resonance spectroscopy (^{1}H-MRS) has been applied to provide insight into the integrity of neurons and into the progression of upper motor neuron pathology in human ALS. However, all *in vivo* MRS applications exhibit metabolite quantification problems due to sensitivity limitations for certain metabolites in the lower mmolar range, e.g. γ – amino butyric acid (GABA) and for small brain structures, such as lower brainstem and cervical spinal cord, with early lower motor neuron involvement in ALS.

Objectives: The objective of our study was to determine how well spectroscopy markers can detect cerebral and spinal pathologic features when there is no or minor neuronal cell loss, and how modifications in metabolite concentrations evolve in time and space.

Methods: Age-dependent alterations in the cerebral and spinal metabolic profile in the mouse model of ALS overexpressing the mutated human G93A-superoxide dismutase-1 (G93A-SOD1) were determined by high-resolution MRS of tissue extracts at 14.1 Tesla. Both non-transgenic mice (control mice) and transgenic mice over-expressing the non-mutated human SOD1 (tg-SOD1) served as controls.

Results: In the spinal cord, the loss of NAA (N-acetyl aspartate) was detectable as early as 34 days postpartum and in the brainstem 75 days postpartum, both before a significant loss of motor neurons and the onset of clinical symptoms become evident, $\sim$ day 90 in this particular ALS model. Since the synthesis of NAA is closely correlated with the mitochondrial energy metabolism we determined in the current work also the putative involvement of the respiratory chain in brain mitochondria of day 40 and day 90 G93A-SOD1 mice. We found in brain mitochondria of day 40 SOD1 mice a selective decrease in complex IV enzyme activity, long before any symptoms arose. Therefore the assumption is supported that alterations in NAA concentrations may also reflect mitochondrial integrity. In addition, correlation analyses between the decreased levels of NAA, glutamate, glutamine, and aspartate but also the inhibitory amino acid GABA in spinal cord and brain stem indicate that factors which modulate the levels of these substances are closely interrelated.

Conclusions: In summary, high-resolution MRS identified multiple age-dependent metabolic alterations mainly in the spinal cord and brainstem of G93A-SOD1 mice

compared with age-matched tg-SOD1 and control mice. An important aspect of this finding is that the metabolite changes can be detected before the onset of clinical signs and even before significant neuronal cell loss occurs. ^{1}H-MRS seems to provide surrogate markers for an early disease detection, for monitoring the progression, and for evaluating a treatment response. The availability of high field strengths may allow for an advanced application of *in vivo* ^{1}H-MRS in different CNS regions of patients with ALS to evaluate metabolites such as NAA or the glutamate–glutamine system.

P181 THE ROLE OF PROGRANULIN IN NEURONAL DEVELOPMENT AND MAINTENANCE

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Background: It has recently been discovered that autosomal dominant mutations within the progranulin gene cause an early onset form of frontotemporal dementia associated with ubiquitin neuronal inclusion bodies (FTDU) (1). Ubiquitin-positive inclusions are associated with other neurodegenerative disorders including amyotrophic lateral sclerosis (ALS). There is evidence of histological and clinical overlap between ALS and FTDU, suggesting common pathogenic mechanisms (2).

Objectives: Using both *in vivo* (mouse, zebrafish) and *in vitro* (cultured NSC-34 cells and primary mouse motor neurons) to determine the role of progranulin gene expression in neuronal survival.

Methods: *Primary motor neuron isolation*: Isolation and culture of primary motor neurons from dissociated spinal cord cultures was carried out as previously described (3). *Zebrafish in vivo progranulin-a knockdown:* Microinjection of progranulin-a specific antisense morpholinos effectively inhibit translation and resulting morphological phenotypes are assessed by whole-mount zebrafish *in situ* hybridization as previously described (4).

Results: *In situ* hybridization reveals strong progranulin gene expression within the dorsal and ventral horn of mouse cortical spinal cord sections as well as cultured primary spinal cord neurons, which include motor neurons. NSC-34 cells were cultured in the presence of recombinant progranulin or engineered stable pcDNA3.1/progranulin transfectants. The addition of recombinant progranulin to NSC-34 cultures resulted in increased cell number at varying concentrations of serum. Similar results were obtained for stable transfectants. Zebrafish progranulin-A morphants exhibit few morphological manifestations prior to 72hpf, with the exception of an overall decrease in animal size. By 120hpf progranulin-A morphants display prominent craniofacial dysmorphogenesis, pericardial oedema and gut dysplasia. Initial analysis of several neural expression patterns including that of *dlx2*, *islet-1* and immunolocalization of Zn8 indicate a restricted set of neural abnormalities, particularly targeting the developing hypothalamus as well as neurons within the retina, sensory ganglia and hindbrain.

Discussion and conclusions: These findings suggest that progranulin, may play critical role in neuronal development and survival and/or proliferation and that progranulin gene expression may be a common link in the spectrum of neurodegenerative diseases that include FTDU and ALS. We hypothesize that progranulin is involved in the homeostasis of neurons and may represent an avenue for novel therapeutic intervention.

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P182 A NOVEL MODEL OF ALS IN ZEBRAFISH TO FUNCTIONALLY CHARACTERIZE MUTATIONS OF THE VAPB GENE

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by the progressive and selective degeneration of motor neurons. While a familial component characterises approximately 10% of ALS cases, relatively few of these can be attributed to an identified gene. A missense mutation (P56S) in the vesicle-associated membrane protein B (*VAPB)* gene has been identified at the *ALS8* locus (1). However, the molecular features and function of VAPB, as well as the pathogenic mechanisms leading to motor neuron death in ALS patients, are not yet understood.

Objectives: To better understand the toxic function of the VAPB gene product, and of the molecular pathways that lead to motor neuron death, through a novel model of ALS. Also, to determine whether other mutations of the VAPB gene are present in familial or sporadic ALS patients, and to functionally characterize these mutations in this zebrafish model.

Methods: We screened all six exons of the *VAPB* gene in a large cohort of sporadic and familial ALS patients in order to identify novel mutations. Then, to perform loss-of-function experiments, we generated anti-sense morpholino oligonucleotides (AMO) complimentary to the region of

translational initiation of the *VAPB* gene. Alternatively, to overexpress the mutant *VAPB* gene in zebrafish, we cloned it in a pCS2^{+} plasmid. Mutations were incorporated by site-directed mutagenesis.

Results: A missense mutation (A145V) and a 3 base-pair deletion (S160del) were detected in a sporadic and a familial patient respectively. Knock-down of the *VAPB* gene by AMO in zebrafish led to abnormalities in development (30%), swimming (30–40%), and axonal guidance as well as decreased firing of motor neurons. Sensory and interneurons were unaffected.
Expression of human VAPB rescued the swimming phenotype in zebrafish, while expression of a *VAPB* construct containing either of the A145V or S160del mutations, as well as the published P56S mutation (1), failed to rescue. Over-expression of mutant VAPB alone failed to induce a motor neuron disorder, indicating that these mutations lead to loss of function in the *VAPB* gene.

Discussion: We performed a functional characterization of *VAPB* gene, leading to a specific motor neuron disorder in zebrafish. We are presently testing whether overexpression of mutant ALS1 (SOD1) or a knock-down of *ALS2* (alsin) leads to motor neuron deficits in zebrafish embryos. These novel *in vivo* models of ALS both enhance the understanding of disease pathogenesis and propose pharmaceutical treatment of the disorder.

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P183 EXCITOTOXIN-INDUCED CHRONIC MOTONEURON DEGENERATION IN CHICK EMBRYO IS LINKED TO PROTEIN RETENTION IN SECRETORY PATHWAYS, AUTOPHAGY AND DEREGULATION OF NEUROMUSCULAR INNERVATION

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We previously showed that *in ovo* application of NMDA from embryonic day (E) 5 to E9 in the chick embryo, results in selective damage to spinal cord motoneurons (MNs) that undergo long-lasting degenerative changes that may be similar to those occurring in MN disease (1,2). This contrasts with a single application of NMDA on E8, or later, which induces massive necrosis of the whole spinal cord (3).
Chronic MN degeneration after NMDA implies altered protein processing within secretory pathways and late activation of autophagy that we demonstrate here by electron microscopy and the redistribution of molecules such as beclin-1 and LC3. Chronic NMDA treatment also results in a transient (between E6 and E10) incompetence to develop naturally occurring or experimentally-induced apoptotic programmed cell death. However, in older embryos (E16), degenerating MNs are sensitized to die after limb ablation (axotomy) and accumulated hyperphosphorylated neurofilaments.
How intracellular calcium handling is affected in degenerating MNs after chronic NMDA treatment was analyzed in acutely dissociated MNs from chick embryos that were previously chronically treated *in ovo*. After loading with Fura-2, a delayed deregulation of intracellular Ca^{2+} was seen in degenerating chronic-excitotoxic MNs when a transient Ca^{2+} influx was induced by a pulse of glutamate in the bath. Moreover, in MNs from chronic NMDA treated embryos an enlargement of thapsigargin-sensitive Ca2+ stores was also detected.
The consequences of NMDA treatment in neuromuscular innervation were also studied. It was found that, in sartorius-innervating MNs, the neuropeptide CGRP is accumulated in somas, peripheral axons and neuromuscular junctions after chronic NMDA treatment. This pattern was not observed in embryos paralyzed by chronic administration of curare. Intramuscular axonal branching was also severely altered after NMDA, as it was shown in whole-mount preparations of leg muscles immunostained with beta-tubulin. Moreover, clustered AChRs in developing muscle fibers were found increased in size after NMDA, whereas the number of neuromuscular synapses was higher than control after either NMDA or curare. Due to the lack of experimental *in vivo* models for sporadic ALS, the paradigm may provide a new experimental system to understand the pathophysiology of chronic excitotoxicity in the neuromuscular system.

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P184 MOTOR UNIT NUMBER ESTIMATION AS A MEASUREMENT OF MOTOR DYSFUNCTION IN A MOUSE MODEL OF FRONTOTEMPORAL DEMENTIA/ALS

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Background: Motor unit number estimation (MUNE) has been shown to reliably correlate with motor weakness and neuronal loss in transgenic animal models of ALS carrying SOD-1 mutations (1). The recently developed P301L tau transgenic model of frontotemporal dementia/ALS (FTD/ALS) shows similar clinical features of progressive motor weakness as the SOD-1 mice (2). The pathophysiology of motor neuron degeneration in the spinal cord is characterized by accumulation of phosphorylated tau and neurofibrillary tangle formation in the P301L tau mouse.

Objectives: To establish that MUNE can be applied to follow the development of motor weakness in the P301L tau mouse model of FTD/ALS.

Methods: Biweekly MUNE of the gastricnemic muscle was used to follow the development of motor weakness in the P301L tau mouse. Immunohistochemistry of the lumbar spinal cord during the disease course was used to detect the appearance of phosphorylated tau and tangles in spinal cord motor neurons. The events of neurodegeneration were compared to the MUNE results and the weight loss of the animals as the disease progressed.

Results: Immunohistochemistry using antibodies against phosphorylated tau show that tau aggregates start to accumulate early (at 3–4 months of age) during the disease course of the P301L tau animals. Starting at age 9 months, we observed loss of lumbar motor neurons. We observed drop out of motor units only at about 12 months of age, at the same time when weight loss becomes noticeable in the P301L tau mice and the hind leg extension is reduced when the mouse is grabbed by its tail. Whereas weight loss occurred at an even rate over 40 days with an average weight loss of about 6% per week, the loss of motor units was more rapid as previously reported in SOD-1 mutant mice.

Discussion and conclusions: This is the first report of a MUNE study in P301L tau mice. We demonstrate that the loss of motor units occurs concomitant with the first signs of motor weakness and weight loss. MUNE appears to be a useful marker of motor unit dysfunction in the P301L tau mouse model of FTD/ALS.

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P185 GAIT, LOCOMOTION AND COGNITION IN A MOUSE MODEL OF ALS-PDC

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Background: Amyotrophic lateral sclerosis-parkinsonism-dementia complex (ALS-PDC) is a neurodegenerative disorder where motor neuron disease may co-occur with parkinsonism and cognitive deficits. ALS-PDC has been linked to the exposure to toxins contained in the seeds from the cycad plant, a former traditional food staple in Guam where the disease was epidemic. An ALS-PDC animal model has been developed by chronically feeding mice with washed-cycad flour. These mice show behavioural and neuropathological evidence of neurodegeneration.

Objective: The objective was to analyze gait, locomotion, social interaction and object recognition in cycad-fed mice and test previously unexplored aspects of motor and cognitive functions in this model.

Methods: Mice were fed washed cycad flour (1 g/day) for a prolonged period of time and subjected to weekly tests of gait and locomotion. Cognitive function was assessed as the ability of the mice to recognize and remember conspecific individuals and familiar objects.
Gait: A ventral plane videography system allowed the measurement of a number of variables, including: length, frequency, and variability of stride; width, duration and width variability of stance, swing duration, braking duration, area and angle of the paws, among others.
Locomotion: The spontaneous activity of the mice in an open field was analyzed with the aid of a computerized video tracking system. The measured variables included: distance moved, velocity, duration of movement, heading angle, turn angle, angular velocity, meander, frequency of rearing, and frequency and duration of visits to the inner area of the arena.
Social Interaction: Social interaction was evaluated as the ratio of time spent in the vicinity of a cage with and without a foreign mouse. Social memory was evaluated as a function of decreased social interaction during a second visit, 1 or 4 days later, to the foreign mouse.
Object Recognition: Object recognition and memory was evaluated as a function of decreased exploration of a familiar object during a second visit to an arena containing a familiar and an unfamiliar object.

Results: Cycad-fed animals showed a transient, but significant, deficit in their gait pattern, as revealed by increased paw angle. However, spontaneous locomotor activity variables did not show any difference between cycad-fed and control animals. Subtle deficits in tasks of social recognition were also transiently detected, whereas object memory seemed to be preserved.

Discussion and conclusion: The spontaneous recovery of the deficits suggests the action of compensatory functional or morphological mechanisms. Nevertheless, the fact that an increased paw angle has been also observed in mutant SOD G93A mice, indicates that this is a useful gait variable for the detection of early motor deficits in ALS models. The presence of social recognition deficits might be related to impairment in olfactory or limbic functions, which would reproduce similar findings in human ALS-PDC.

P186 TIMELINES OF BEHAVIOURAL, ANATOMICAL AND BIOCHEMICAL CHANGES IN THE CNS OF AN ANIMAL MODEL OF ALS-PDC OF GUAM

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Background: An almost classical form of ALS, ALS-parkinsonism dementia complex (ALS-PDC) was first described on Guam in years immediately after WWII. Incidence levels were up to 400 times higher than in North America. The disease peaked in the 1960s then began to decline to a current level similar to that of the industrialized world. The clearest epidemiological link to the disease was consumption of flour made from the seeds of a type of cycad, *Cycas micronesica*. Disease levels declined in step with decreased use of cycad as food. Intensive studies identified various novel amino acid toxins in cycad seeds, including MAM, BOAA, and BMAA. None of these were able to induce an ALS phenotype in animal models. We re-examined the ''cycad hypothesis'' by feeding washed cycad seed flour in which the above toxins had been removed to CD-1 adult male mice for a prolonged period while testing motor and other behaviours. Post sacrifice, we examined the CNS using histological and biochemical methods. Cycad-fed mice displayed an early ALS phenotype, including deficits in leg extension and gait; motor neurons were lost in ventral spinal cord and elsewhere in the CNS. The impacts at the behavioural and anatomical levels were progressive. Similar experiments in which one of the active cycad neurotoxins, a sterol glucoside (β-sitosterol β-D glucoside, BSSG) was fed to mice showed qualitatively similar outcomes.

Objectives: To determine the temporal progression of the ALS phenotype at behavioural, systems, cellular, and biochemical levels.

Methods: Adult male (>5 mo old) CD1 or C57/Bl6 mice were used. Motor tests included leg extension, rotarod, gait, wire hang, detailed gait analysis, social interaction, and object memory. Mice were sacrificed at various time points following the onset of cycad or BSSG feeding and processed for histological analysis. Neuromuscular junction (NMJ) integrity was assessed by co-labelling of nicotinic receptors (bungarotoxin) and nerve terminals (neurofilament/SV2) in the gastrocnemius muscle. *In vitro* studies using NSC-34, a motor neuron-derived cell line, exposed to sterol glucosides complemented the *in vivo* work.

Results and Discussion: Behavioural results are presented in an accompanying abstract and poster (P185). Lumbar ventral cord showed significant losses of motor neurons accompanied by microglial proliferation early during cycad treatment. Neither spinal cord astrogliosis nor NMJ denervation were detected at this time point. *In vitro*, treatment with synthetic sterol glucosides reduced NSC34 cell viability in a dose- and time-dependent fashion. Treated NSC34 cells showed morphological alterations, including the formation of Tau-positive TDP-43-negative axonal beading.

Conclusions: Cycad neurotoxins such as BSSG can reproduce aspects of Guamanian ALS-PDC, with the first events apparently involving the motor system in which motor neurons and microglia are the earliest cells affected.

P187 THE SMA DISEASE MOUSE MODEL RESOURCE AT THE JACKSON LABORATORY: THE EFFECTS OF GENETIC BACKGROUND ON PHENOTYPE

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Mouse models are critical tools to study the basic pathogenesis of Spinal Muscular Atrophy and can be used to develop new therapies. There are numerous mouse models for SMA of differing severities in the published literature, each with strengths and weaknesses for particular experimental usage. Many additional strains are being developed that will enhance the knowledge gained by existing models; including new conditional models that allow regulation of expression of disease-causing genes, along with GFP and Cre-expressing transgenes to accompany these new models. The SMA Mouse Model Repository at The Jackson Laboratory is a central resource for archiving and distributing SMA models at a high health status to the Scientific Community.

Although mouse models are acknowledged to be critical for SMA research, there are many obstacles to their efficient use, including insufficient knowledge of the effects of genetic background on mutant phenotypes. It is widely known that different genetic backgrounds can harbor modifier alleles that often change the severity and/or onset of a disease phenotype. Equally true is that some disease phenotypes show variability as a result of environmental influences. In addition to distributing mouse models of SMA to the scientific community, The Jackson Laboratory is looking at the effects of genetic background on phenotype for many SMA strains. Two of the more widely distributed models at The Jackson Laboratory are those originating from the laboratory of Dr. Arthur Burghes: Stock number 005024 FVB.Cg-Tg(SMN2)89Ahmb *Smn1*^{tm1Msd}/J is the more severe model resembling Type 1 SMA while stock 005025 FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb *Smn1*^{tm1Msd}/J, also referred to as the delta7 line, resembles a Type 11 intermediate phenotype. Both of these lines show some degree of variability in their survival of mutant animals. At the time of importation to TJL, both of these strains had been backcrossed to FVB/NJ 5 to 6 generations by the Burghes Laboratory making them primarily FVB/NJ in their genetic background, although not fully congenic. We have performed genome scans on both the currently distributed 005024 and 005025 stocks to determine the degree of heterogeneity in their genetic backgrounds. We have also continued the backcross of both of these lines to the N10 generation and present data that

compares the phenotype of the fully congenic lines with the phenotype of partially congenic lines.

P188 ANALYSIS OF MUTANT SMN AND CHARACTERIZATION OF AXONAL COMPLEXES

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Loss of the *Survival Motor Neuron 1* (*SMN1*) gene, with retention of the *SMN2* gene, causes the genetic disease, Spinal Muscular Atrophy (SMA). Despite ubiquitous expression of SMN, mutation and deletion of the *SMN1* gene locus primarily affects spinal motor neurons. The Tudor domain of SMN binds Sm proteins, which form the core of spliceosomal U snRNPs. Current evidence indicates that SMN may have a function outside of splicing, as SMN is found in the axon and growth cone of developing motor neurons without Sm proteins. Furthermore, it was found that certain mutations of SMN have characteristics that do not correlate with ability to rescue axonal pathogenesis in a zebrafish model of SMA. An axonal function of SMN may explain the specificity of SMN for motor neuron growth and maintenance.

Transgenic mice have been generated for the mutants SMN(A111G) and SMN(VDQNQKE). SMN(A111G) is a mutant that performed well in GST-fusion binding assays and snRNP biogenesis, but failed to rescue the abnormal branching and truncation of neurons in zebrafish. Whereas, the SMN(VDQNQKE) performed poorly in binding assays, but rescued in the zebrafish. Eleven lines of SMN(A111G) were obtained and to date, 5 lines express the transgene. Ten lines of SMN(VDQNQKE) were obtained and 4 lines express the transgene. Both the SMN(A111G) and the SMN(VDQNQKE) expressing lines are bred to mSmn knockout mice as well as bred onto the SMA background (SMN2; mSMN KO) to assess the effect of the transgene on survival of SMA mice.

Stable cell lines have been generated for SMN(wt)-FLAG, SMN(Q282A)-FLAG and SMN(A111G)-FLAG. MN1 cells were transfected with each construct and at least 3 stably expressing lines have been obtained for each construct. Immunolocalization and FLAG-tag pull downs will be performed to capture motor neuron complexes and compare the profiles of the different mutants.

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THEME 8 IN VITRO EXPERIMENTAL MODELS

P189 IMPACT OF HFE POLYMORPHISMS ON CELLULAR GLUTAMATE REGULATION

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Background: Glutamate excitotoxicity is considered to be a major cause of motor neuron death in ALS. Either excessive glutamate secretion into the synaptic cleft or deficient glutamate uptake into surrounding astrocytes or neurons can contribute to glutamate excitotoxicity. Five independent published studies have reported the frequencies of HFE polymorphisms in ALS patients compared to various control groups. A meta-analysis of these studies shows that possession of at least one H63D allele is associated with a 26% increased risk of developing ALS. We examined the role this polymorphism may play in cellular glutamate regulation using the human neuroblastoma cell line, SH-SY5Y, stably transfected with different genetic variants of HFE.

Objective: Determine the role of different HFE polymorphisms on cellular regulation of glutamate.

Methods: The impact of the HFE polymorphisms on the glutamatergic profile was obtained. Glutamate was measured in cell culture media following 48 hours of incubation. Additionally, we determined the expression of gene transcripts for the vesicular glutamate transporters VGLUT1 and VGLUT2. Expression of the excitatory amino acid transporters, EAAT1, EAAT2, and EAAT3 was determined by quantitative RT-PCR and Western blotting. Sodium-dependent uptake of L-[3,4-^{3}H]-glutamate was measured in the cells over a 30 minute interval in the presence and absence of inhibitors of the EAATs. To begin to examine how the HFE polymorphisms could impact intervention strategies in ALS, the cells were exposed to minocycline, a drug that delays disease onset and prolongs survival in rodent models of ALS, for 48 hours prior to measuring glutamate uptake.

Results: Quantitative RT-PCR showed expression of VGLUT1 and VGLUT2 in each of the cell lines. Cells expressing H63D HFE secreted approximately 31% more glutamate ($p < 0.05$) than cells expressing wild type (wt) HFE. Cells expressing C282Y HFE secreted approximately half as much glutamate as the wt HFE cells ($p < 0.001$). Over a 30 minute interval, cells expressing H63D HFE transported 59% less glutamate than wt HFE cells ($p < 0.05$), whereas cells expressing C282Y HFE transported 49% more glutamate than wt HFE cells ($p < 0.001$). Each of the cell lines was shown to express EAAT1, EAAT2, and EAAT3 by PCR and Western blotting. All sodium-dependent glutamate transport was inhibited by THA, an EAAT1-5 inhibitor, and L-SOS, an EAAT1/3 inhibitor. DHK, a selective EAAT2 inhibitor, did not alter glutamate transport. Forty-eight hours of 25 μM minocycline treatment decreased glutamate uptake in the wt HFE cells by 77% ($p < 0.001$), but did not significantly alter glutamate uptake in the other cells.

Discussion and conclusions: We have previously demonstrated that the different genetic variants of HFE alter cellular characteristics. In this study we focus on those cell functions that may be relevant to ALS. These data suggest that H63D genetic variants may play a role in the etiopathogenesis of ALS by altering glutamatergic uptake and release. The data also strongly suggest that pharmaceutical interventions for ALS may have different effects based on the type of HFE polymorphism that is present. Future studies will be aimed at elucidating the cellular functions of HFE and the mechanisms of altered cellular glutamate regulation.

P190 CANNABINOID RECEPTOR ACTIVATION PREVENTS TNFα-INDUCED SURFACE DELIVERY OF GLUR2-LACKING AMPARS AND PROTECTS NEURONS FROM EXCITOTOXIC STRESS

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Recent reports indicate early changes in hippocampal AMPAR levels during disease progression in the *SOD1^{G93A}* mouse model of ALS/MND (1). The cause of this early elevation of AMPARs in brains of mice is not yet known, but glial-derived TNFα causes a rapid surface localization of calcium permeable AMPARs in hippocampal cultured neurons (2). TNFα is a recently discovered modulator of AMPAR surface delivery that has recently been shown to be highly relevant to homeosynaptic scaling in development and to the vulnerability of neurons to glutamatergic excitotoxicity after injury or during disease. Another class of neuronal receptors on hippocampal neurons that has been shown to be relevant to the neuron's excitotoxic vulnerability is the inhibitory cannabinoid receptors. The objective of this study was to determine whether cannabinoid receptor activation could prevent TNFα-induced surface delivery of AMPAR and resulting excitotoxicity. Here we show that TNFα potentiates excitotoxic neuron death in cultured hippocampal neurons in a standard *in vitro* excitotoxicity assay. Furthermore we show that TNFα -induced surface delivery of GluR2-lacking AMPARs and the excitotoxic

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potentiation is blocked by cannabinoid receptor activation. We employ microscopic assays to examine neurons with or without TNFα exposure and we quantitatively measure the distribution of AMPARs that are surface vs. internal. These results provide a novel picture of the cellular mechanisms in control of surface localization of calcium permeable AMPARs following acute injury or during neurodegenerative disease and mental health disorders. A greater understanding of this process may aid in the development of neuroprotective strategies aimed at counteracting the increase in excitotoxic vulnerability caused by excessive TNFα exposure after injury or during prolonged neurodegenerative disease, such as ALS/MND.

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P191 ANALYSIS OF NEUROPROTECTIVE EFFECTS OF VALPROIC ACID AND ANALOGOUS COMPOUNDS IN A RAT MOTONEURON MODEL OF EXCITOTOXICITY

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Background: Valproic acid (VPA) is widely used as an antiepileptic drug. Recently, it has also been successfully applied in cellular and animal models of chronic neurodegeneration. Its neuroprotective capacities are attributed to its inhibition of histone deacetylases. The anticonvulsive potential has mainly been related to an enhancement of GABAergic neurotransmission and interactions with voltage gated sodium channels.

Objective: The effects of the three compounds tested were analysed on the level of a cell culture model of excitotoxic neuronal death, on motoneuron calcium transients and electrophysiological activity, and regarding their functional interactions with AMPA-type receptors and further synaptic channels. We aimed to evaluate the neuroprotective properties of VPA and two analogs of this substance (3- propylheptanoic acid, (R)- pentyl-4-Yn-VPA) and trace the molecular mechanism of action focusing on effects on cell and membrane excitability.

Methods: In our toxicity experiments we exposed cultured rat embryonic motoneurons for 36 hours to kainate, the tested compound, a combination of both or to medium as control conditions. After incubation, cells were fixed and double-stained with antibodies to ß- III- Tubulin (all types of neurons) and SMI 32 (specific for motoneurons) to determine the total number of neurons and the number of motoneurons. We used the ratio of SMI 32 positive cells to all neurons to compare the different conditions. FURA-2 AM calcium imaging was used to monitor cytosolic calcium transients in individual neurons. The patch clamp technique was combined with a fast application system for test solutions.

Results: Under control conditions, the fraction of motoneurons in relation to all neurons was about 50%. In presence of kainate, this fraction was reduced to about 15%. Additional application of VPA enhanced cell survival by 33%. In contrast, the combination of kainate and 3-pentyl-heptanoic acid further decreased the amount of motoneurons by 50%. R-pentyl-4-Yn-VPA had no significant effect on cell counts. In calcium imaging studies motoneurons showed high intracellular calcium transients after short pulses of kainate. The amplitude of the calcium transients was reduced in the presence of VPA, but increased by application of R-pentyl-4-Yn-VPA. In a first set of experiments with different subtypes of recombinant AMPA receptors we did not identify significant interactions on that level while there was a moderate enhancement of GABAergic currents in the presence of VPA.

Conclusion: VPA showed a protective effect on motoneurons exposed to excitotoxic stimuli and reduced kainate-induced calcium transients. In contrast, 3-pentyl-heptanoic acid even increased excitotoxic cell death while R-pentyl-4-Yn-VPA was not protective against excitotoxicity but led to an increased ratio of motoneurons compared to medium alone. Since a direct effect of VPA and its analogous compounds on AMPA-type glutamate receptors tested could be ruled out, we suppose that the effects found in our toxicity and calcium imaging experiments were mediated by other players of synaptic transmission and/or intracellular pathways that will be further investigated and could provide a molecular basis for the development of more specific therapies for neurodegeneration due to excitotoxicity.

P192 GLUTAMATE ENHANCES MITOCHONDRIAL ATP PRODUCTION AND ROS GENERATION IN NONSYNAPTIC BRAIN AND SPINAL CORD MITOCHONDRIA OF THE WILD TYPE AND SOD1 MUTANT RATS

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Background: According to current paradigm (1), neuronal mitochondria of adult mammals utilize pyruvate as the major source of energy. Upon activation of neurons, reuptake of glutamate into glial cells stimulates aerobic glycolysis, and thus astrocytes provide neurons with additional amounts of lactate for oxidation. Besides pyruvate, glutamate is also a good respiratory substrate for the brain (BM) and spinal cord mitochondria (SCM). About 85–90% of synapses are excitatory, and 10–15% are

inhibitory, with glutamate and GABA as neuromediators. GABA is degraded to succinate in postsynaptic mitochondria. Glutamate is rapidly removed from the synaptic cleft by various glutamate transporters: GLT-1 is localized only in glia, GLAST was found both in neurons and glia, and EAAC1 is confined to postsynaptic elements only (Reviewed in 1). Thus, mitochondria, located at synaptic junctions, upon activation may oxidize a mixture of substrates.

Objectives: To study how BM and SCM from wild type (WT) and SOD1 mutant rats (MR) respond to different combinations of respiratory substrates, namely pyruvate, glutamate, succinate and malate.

Methods: Nonsynaptic BM and SCM were isolated as described in (2). Respiration and ROS generation were measured as in (2). All data were normalized to BM or SCM oxidizing pyruvate and malate as substrates.

Results: With pyruvate, glutamate and malate, the rate of oxidative phosphorylation (OXPHOS) increased by 30–51% and uncoupled respiration by 70–80%. In State 4, the rate of ROS generation increased by 50–70% in WTBM and 1.5–2 fold in WTSCM. With pyruvate, glutamate and malate as substrates, ROS generation increased 1.5–2 folds with MRBM, and 3–4 folds with MRSCM. The effects on ROS generation were much more pronounced with MRSCM. The presence of M mitigated production of ROS but did not affect significantly OXPHOS.

Conclusions: In the postsynaptic neurons glutamate, besides its actions on specific receptors, also stimulates mitochondrial ATP production. Thus during increased neuronal activity glutamate not only increases the supply of lactate by astroglia, but also increases the rate of OXPHOS in postsynaptic neurons. However, in a resting cell, glutamate may significantly increase ROS generation, and this effect was much higher in SCM, particularly in MRSCM. These new data open a new pathway in understanding mechanisms that govern specificity of neurodegeneration. We suggest that increased ROS generation by SCM during activation of motor neurons may cause early dysfunctions and cell death, which begin at the peripheral neuronal and neuromuscular junctions.

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P193 MECHANISM(S) OF MUTANT SOD1-MEDIATED MITOCHONDRIAL DYSFUNCTION

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Background: Mitochondrial abnormalities and activation of the mitochondrial apoptotic pathway are both characteristic features of mutant SOD1-mediated motor neuron death. Numerous studies suggest that mutant SOD1-mediated localization and aggregation within the mitochondria may mediate mutant SOD1-induced motor neuron death. It is unclear whether the aggregated forms of mutant SOD1 are per se directly injurious to the mitochondria or whether they cause toxicity by forming harmful interactions with other mitochondrial proteins.

Objectives: The goal of this study is to define the mechanism(s) by which unstable, mutant SOD1 damages the mitochondria. We set up a series of *in vitro* experiments to determine whether aggregated mutant SOD1 is per se directly toxic or whether it requires interaction with other mitochondrial proteins.

Methods: *In vitro experiments:* Mitochondria isolated from brain, spinal cord or liver were incubated with various forms (monomer, dimer or oligomer) of recombinant mutant SOD1s. Mitochondrial integrity was then assessed by a) measuring mitochondrial swelling spectrophotometrically and b) measuring cytochrome C release into the cytosol with Western blot analysis. *Cell culture-based experiments:* Cells were transfected with various SOD1 cDNAs (WT or mutant SOD1), co-immunoprecipitation experiments on isolated mitochondria were performed to identify potentially harmful mutant SOD1-binding partners.

Results: We present *in vitro* evidence that mutant SOD1 directly damages the mitochondria by triggering mitochondrial swelling and release of cytochrome C and that to fully become toxic mutant SOD1 requires an aberrant interaction with other mitochondrial proteins.

Conclusions: These data suggest that mutant SOD1-mediated mitochondrial toxicity involves a ''double-hit'' mechanism in which misfolded mutant SOD1 recruits toxic partner(s) to damage the mitochondria.

P194 ROLE OF NORMAL AND MUTANT SOD1 PROTEINS AND THE INVOLVEMENT OF MITOCHONDRIA IN APOPTOTIC SIGNALLING IN MOTOR NEURON-LIKE CELLS

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Background: Amyotrophic lateral sclerosis (ALS) is associated with mutations of the anti-oxidant protein, Cu,Zn-superoxide dismutase 1 (SOD1). Aggregation of SOD1 and its deposition into intracellular inclusions causes cytotoxicity particularly in motor neuron-like cells. Previous studies have made links between SOD1 deposition and mitochondrial dysfunction, but until now there has not been satisfactory molecular definition of the precise pathways of cell death.

Objectives: To characterize the induction of apoptosis and the role of inclusions of mutant SOD1 in cultured human motor neuron-like cells (NSC-34).

Methods: NSC-34 cells were transfected with wildtype (WT) and mutant (A4V) SOD1-EGFP constructs. Immunohistochemistry was performed to study recruitment to mitochondria of the proapoptotic protein, Bax, and its activation, redistribution of cytochrome C from mitochondria and activation of caspase-3. Nuclear morphology was studied using DAPI staining. Imaging of cells was carried out by confocal microscopy.

Results: In cells expressing tagged WT SOD1, the fluorescent protein was dispersed through the cells without inclusions. Although fluorescence was dispersed in the majority of cells expressing tagged mutant SOD1-A4V, in a minority there were cytoplasmically localized inclusions of SOD1-A4V. First, we established whether expression of SOD1 induced apoptosis, monitoring changes in nuclear morphology after DAPI staining. Only in cells expressing SOD1-A4V in inclusions (but not dispersed) was there significant apoptosis. Indeed, in the 20–40% of inclusion-positive cells scored as being apoptotic, the nuclei were invariably so damaged the DAPI stain had become almost imperceptible. Second, we examined the susceptibility to apoptosis induced by staurosporine (STS). Cells containing inclusions of SOD1-A4V were exquisitely sensitive to apoptosis by STS. However, cells expressing dispersed forms of SOD1 (either WT or A4V) were strongly protected against apoptosis, relative to non-transfected controls. Third, we addressed the participation of mitochondria in apoptosis induced by mutant SOD1 inclusions. In cells expressing SOD1-A4V, mitochondrial apoptotic events, including Bax recruitment and activation, as well as cytochrome c release to cytosol, took place in most cells undergoing apoptosis. On the other hand, in cells expressing dispersed SOD1 (WT and A4V), those mitochondrial events were extensively suppressed. In addition, caspase-3 activation, which is downstream of mitochondria in the apoptotic signalling pathway, was also upregulated in cells containing inclusions of SOD1-A4V. Caspase-3 activation was suppressed in cells expressing dispersed forms of SOD1 (WT and A4V).

Discussion and conclusions: The results demonstrate the character of cytoplasmically dispersed SOD1 (even in mutant form) as cytoprotective. Thus, we showed that dispersed SOD1 acts upstream of mitochondria to prevent an apoptotic response. However, inclusions of mutant SOD1 are highly cytotoxic, inducing apoptosis in large measure via the mitochondrial pathway. These findings emphasize two divergent aspects of SOD1 biology: the cytoprotective effect of a normal protein that is countered by its profound cytotoxicity when mutant SOD1 forms inclusions. Understanding this duality should provide clues as the molecular pathology of ALS and its possible treatment.

P195 MUTANT SOD1 DIMINISHES MITOCHONDRIAL CA^{2+} BUFFERING

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Selective motor neuron death in amyotrophic lateral sclerosis (ALS) has been linked to excessive Ca^{2+} influx through Ca^{2+}-permeable AMPA receptors. The fate of Ca^{2+} after entering the motor neuron is not well established. Due to the lack of Ca^{2+} binding proteins, it has been suggested that mitochondria play an important role in this buffering process. We have previously shown that a short stimulation of AMPA receptors in combination with inhibition of the mitochondrial uniporter by RU-360 caused immediate $[Ca^{2+}]_c$ overload in primary motor neurons. Moreover, repetitive short stimulations of the AMPA receptors caused a slowing of the decay of the Ca^{2+} transient and a gradual increase in peak and baseline $[Ca^{2+}]_c$ in motor neurons, but not in other neurons. This was due to the saturation of the mitochondrial buffer capacity. Using mitotracker, we discovered that the mitochondrial density was lower in motor neurons. These data indicate that in combination with an excessive influx of Ca^{2+} through the Ca^{2+}-permeable AMPA receptors, motor neurons have a lower mitochondrial capacity to buffer large volumes of Ca^{2+} ions. This is partly due to a reduced mitochondrial density per volume compared to non-motor neurons. In the present study, we studied the effect of the presence of mutant SOD1 on mitochondrial Ca^{2+} buffering. Mitochondrial tagged aequorine was used to measure mitochondrial Ca^{2+} concentration upon bradykinine induced Ca^{2+} release from the endoplasmic reticulum. Using aequorine instead of dyes has the advantage that it doesn't interfere with the Ca^{2+} concentration and hence energy production of the cell. We observed no difference in bradykinine induced mitochondrial Ca^{2+} loading between neuroblastoma cells overexpressing mutant and WT SOD1. Mitochondria of cells overexpressing different mutations, G37R, G41D

and G85R showed reduced Ca^{2+} buffering compared to WT overexpressing and untransfected cells. The largest reduction in Ca^{2+} buffering was seen in G37R overexpressing cells followed by G41D and G85R. This reduced buffering was not due to enzymatic activity of the mtSOD1 as both active (G37R and G41D) as well as inactive (G85R) forms of mtSOD1 displayed a reduced loading of Ca^{2+}. We found that the affinity of the uniporter was similar in non transgenic, WT and mutant cell lines. In conclusion, we established that mitochondria play a crucial role in buffering Ca^{2+} entering the motor neuron through Ca^{2+}-permeable AMPA receptors. Moreover, mutant SOD1 interferes with the buffering capacity of mitochondria which will lead to excessive cytoplasmic Ca^{2+} and to excitotoxic motor neuron death. In order to elucidate the mechanism by which mtSOD1 interferes with Ca^{2+} buffering in mitochondria, we are currently investigating the mitochondrial release pathways, mitochondrial network and distribution of mitochondria.

P196 EXPRESSION OF MUTANT SOD1^{G93A} INDUCES FUNCTIONAL DEFICITS IN MOTONEURON MITOCHONDRIA

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Background: Although Amyotrophic Lateral Sclerosis (ALS) is regarded as a motoneuron-specific disorder, increasing evidence from both post-mortem ALS tissue and SOD1 mice indicates that non-neuronal cells play a significant role in disease pathogenesis (1–4). It has also been established that some of the earliest pathological changes in SOD1 mice are abnormalities in mitochondrial structure and function (5). Similar changes have also been observed in post-mortem ALS tissue suggesting that mitochondria may be a primary target for damage in ALS.

Objectives: Since glial cells and mitochondrial damage are implicated in ALS pathogenesis, in this study we examined the effect that expression of SOD1^{G93A} within astrocytes has on mitochondrial homeostasis in motoneurons at a cellular level in an *in vitro* co-culture model.

Methods: Primary motoneurons from wild-type (WT) or transgenic mice carrying the SOD1^{G93A} mutation were plated onto a layer of either WT or SOD1^{G93A} astrocytes. Various aspects of mitochondrial function were examined at 7 days *in vitro* using a number of fluorimetric dyes and confocal microscopy. Data were analysed using a one-way ANOVA incorporating a Student Newman Keuls multiple comparison test.

Results: Under resting conditions, SOD1^{G93A} expression in astrocytes induced changes in mitochondrial function in both SOD1^{G93A} and WT motoneurons. In the presence of SOD1^{G93A} astrocytes, mitochondria of both WT and SOD1^{G93A} motoneurons were depolarized ($p < 0.005$). In contrast the mitochondrial membrane potential of SOD1^{G93A} motoneurons co-cultured with WT astrocytes did not differ from WT co-cultures ($p > 0.3$). Furthermore, the presence of SOD1^{G93A} astrocytes was associated with functional alterations in mitochondrial redox state in co-cultured motoneurons, as measured by NADH autofluorescence, suggesting impaired respiration, which may mediate the mitochondrial depolarization observed in these co-cultures. Intra-mitochondrial calcium levels ($[Ca^{2+}]_m$) were also elevated in SOD1^{G93A} motoneurons, however changes in mitochondrial function did not correlate with $[Ca^{2+}]_m$.

Conclusion: These results show that expression of SOD1^{G93A} in astrocytes influences motoneuron mitochondrial function long before SOD1^{G93A} mice develop symptoms. Such early deficits in mitochondrial function induced by SOD1^{G93A} astrocytes, are likely to contribute to the vulnerability of motoneurons to neurotoxic mechanisms involved in ALS pathogenesis.

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P197 ANTEROGRADE TRANSPORT OF MITOCHONDRIA IS SELECTIVELY AFFECTED IN SOD1^{G93A} MOTONEURONS

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Background: Deficits in axonal transport play an important role in Amyotrophic Lateral Sclerosis (ALS) and defects in both retrograde and anterograde transport have been observed in SOD1 mice prior to disease onset (1–4). Indeed, deficits in retrograde transport are one of the earliest pathological changes reported and are even present in embryonic SOD1 motoneurons (4). Mitochondrial defects also occur early in disease, with abnormalities in mitochondrial structure and function reported in both SOD1 mice and post-mortem ALS tissue (5), suggesting that mitochondria may be a primary target for damage in ALS.

Objectives: Axonal transport of mitochondria is likely to be critically important for motoneurons, which require an efficient energy supply at their synaptic terminals at great distance from the cell body. In view of the involvement of both defective axonal transport and mitochondrial abnormalities in ALS, we examined whether expression of $SOD1^{G93A}$ altered the normal trafficking of mitochondria in embryonic motoneurons derived from $SOD1^{G93A}$ mice.

Methods: Primary motoneurons from E13 wild-type (WT) or $SOD1^{G93A}$ mice were cultured for 7–10 days *in vitro*. Cultures were loaded with Alexa 555-TeNT Hc (40nm) and Mitotracker Green (50nm) for 30 minutes at 37°C. Axonal transport of TeNT Hc and Mitotracker was carried out simultaneously by time-lapse confocal microscopy. Tracking of moving carriers and mitochondria was carried out using motion analysis software (AQM tracker).

Results: Consistent with our previous findings, there was a significant deficit in the retrograde transport of TeNT Hc in $SOD1^{G93A}$ motoneurons (4). This finding confirms both the reproducibility of this assay and the validity of our previous results. Analysis of the anterograde mitochondrial transport revealed that in $SOD1^{G93A}$ motoneurons the speed of transport was significantly reduced. In addition $SOD1^{G93A}$ mitochondria had a significantly increased incidence of pausing ($p < 0.05$) compared to WT mitochondria.

Discussion: This study identifies a specific deficit in the anterograde transport of mitochondria in embryonic $SOD1^{G93A}$ motoneurons. It is possible that $SOD1^{G93A}$-induced changes in mitochondrial ultrastructure and function disrupts their recruitment to the anterograde transport machinery. This in turn may alter the normal distribution of mitochondria along the axon resulting in an inadequate energy supply to the distal regions of the axon. These results therefore suggest that impaired anterograde transport of mitochondria may be a primary deficit in ALS.

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P198 TOXICITY OF MUTANT SUPEROXIDE DISMUTASE 1 (SOD1) IN MODELS OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (FALS)

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by a selective loss of both upper and lower motorneurons. Some inherited cases (familial ALS or fALS) are linked to superoxide dismutase 1 (SOD1) mutations, one of the major cellular antioxidant enzymes, whose activity is preserved in most mutant forms.

We have performed analysis of immunocytochemical localization of human SOD1, in vibratome spinal cord sections from transgenic mice expressing either wild type (wt), or mutant G93A-SOD1 (with a Gly to Ala substitution in 93). We found a reduced distribution of mutant SOD1 in nuclei of motorneurons, if compared to wtSOD1. Similar results were obtained with immunofluorecence and sub-fractionation analysis performed on immortalized motorneurons (NSC34 cells) expressing wt or G93A-SOD1. This may be due to the formation of insoluble high MW species of mutant SOD1 that prevent the diffusion of the protein across the nuclear membrane, whereas this diffusion is possible for wtSOD1.

Using reporter plasmids expressing yellow fluorescent protein, carrying a nuclear localization signal (YFP-NLS) or a nuclear exporting signal (YFP-NES), fused with a short degron which address YFP to proteasome degradation, we have analyzed the Ubiquitin-Proteasome-Pathway (UPP), responsible for misfolded protein clearance, in the two subcellular compartments. We found proteasome impairment only in the cytoplasm. The effect of G93A-SOD1 exclusion from nuclei was then analyzed in NSC34 expressing SOD1s, after induction of oxidative stress by H_2O_2 treatment, using the Comet assay. Cells expressing G93A-SOD1 showed higher DNA damage compared to those expressing wtSOD1, possibly because of a loss of nuclear protection. Based on our results showing that G93A-SOD1 is excluded from the nucleus, the effect we have observed could be ascribed to the increased nuclear concentration of free radical species due to a lower clearance in the nucleus. The data obtained suggest that the nucleus may be a target of G93A-SOD1 neurotoxicity in fALS which might arise from an initial misfolding (gain-of-function), generating nuclear deprivation of the active enzyme (loss-of-function in the nuclei), a process that may be involved in ALS pathogenetic process.

P199 PROTEIN DISULFIDE ISOMERASE INHIBITS SOD1 AGGREGATION AND DECREASES ENDOPLASMIC RETICULUM STRESS IN ALS

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Background: Motor neurons selectively degenerate in amyotrophic lateral sclerosis (ALS), a disease that can be caused by numerous familial mutations to the gene encoding superoxide dismutase-1 (SOD1). Although the mechanisms of neurodegeneration remain unclear, aberrant protein misfolding and aggregation are linked with disease severity of SOD1-linked ALS. Recently a role for endoplasmic reticulum (ER) stress has also become apparent in ALS, as well as other neurodegenerative diseases. Our laboratory has previously shown upregulated expression of many ER stress response proteins in mutant SOD1 transgenic rodents, including the molecular chaperone and disulfide bond modulating enzyme, protein disulfide isomerase (PDI). SOD1 possesses an intrasubunit disulfide bond that stabilizes the protein and is important in the formation of the enzymatically active dimer, and evidence suggests that aberrant SOD1 disulfide bonding leads to the formation of cross-linked high molecular weight complexes and insoluble protein aggregates. We therefore hypothesized that expression of PDI could inhibit the formation of these protein aggregates, and could possess neuroprotective properties in ALS.

Objectives: The aim of this study was to examine the effect of PDI expression on intracellular SOD1 misfolding and aggregation. We also aimed to investigate the effect of PDI on expression of ER stress response proteins in ALS models.

Methods: Motor neuron-like NSC34 cell lines with overexpression of either wildtype or mutant SOD1, or PDI, were constructed and studied. Immunocytochemistry, confocal microscopy and immunoblotting were performed to detect protein inclusions and high-molecular weight insoluble protein aggregates, as well as markers of ER stress.

Results: Immunocytochemistry showed that expression of PDI decreased the formation of large mutant SOD1 aggregates, and immunoblotting showed a decrease in the amount of high molecular weight mutant SOD1 species in the presence of PDI. Mutant SOD1 also induced expression of ER stress response proteins, such as CHOP and PERK, in these cell culture models, and this induction was decreased in cells overexpressing PDI.

Discussion and conclusions: Protein aggregation is a hallmark of a number of neurodegenerative diseases, including ALS, and prevention of this feature of disease is a possibility for development of effective therapies. We show here that PDI inhibits the aggregation of mutant SOD1. Similarly, we show that PDI decreases the ER stress response, which if protracted can cause cell death by apoptotic signaling and has been shown to be involved in ALS pathogenesis. These findings warrant further investigation of PDI and the ER stress response as possible therapeutic targets for ALS, as well as other neurodegenerative disorders.

P200 INTERACTION BETWEEN FAMILIAL ALS-LINKED SOD1 MUTANTS AND THE DYNEIN COMPLEX

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive motor neuron death. More than 90 mutations in the copper-zinc superoxide dismutase (SOD1) gene cause a subset of familial ALS. Toxic properties have been proposed for the ALS-linked SOD1 mutants, but the nature of the toxicity has not been clearly specified. Cytoplasmic inclusion bodies containing mutant SOD1 and a number of other proteins are a pathological hallmark of mutant SOD1 mediated familial ALS, but whether such aggregates are toxic to motor neurons remains unclear.

Objective: We identified a dynein subunit as a component of the mutant SOD1-containing high-molecular-weight complexes using proteomic techniques. In this study, we will determine whether mutant SOD1 can interact with the dynein complex, and what the functional significance of such interaction.

Methods: Immunoprecipitation and immunohistology using cultured cells and transgenic animals are the major techniques used in this study.

Results: We further demonstrated interaction and colocalization between dynein and mutant SOD1, but not normal SOD1, in cultured cells and also in G93A and G85R transgenic rodent tissues. Moreover, the interaction occurred early, prior to the onset of symptoms in the ALS animal models and increased over the disease progression. The results also showed that the interaction between mutant SOD1 and the dynein complex is essential to the formation of protein inclusions in cell culture system.

Discussion and conclusions: Motor neurons with long axons are particularly susceptible to defects in axonal transport. Our results demonstrate a direct ''gain-of-interaction'' between mutant SOD1 and dynein, which may provide insights into the mechanism by which mutant SOD1 could contribute to a defect in retrograde axonal transport or other dynein functions. The aberrant interaction is potentially critical to the formation of mutant SOD1 aggregates as well as the toxic cascades leading to motor neuron degeneration in ALS.

P201 ROLE OF P53 IN NEUROTOXICITY INDUCED BY THE ENDOPLASMIC RETICULUM STRESS AGENT TUNICAMYCIN IN ORGANOTYPIC SLICE CULTURES OF RAT SPINAL CORD

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Background: The endoplasmic reticulum (ER) is an intracellular organelle which is important for the folding and maturation of cellular proteins. Various stimuli can disrupt ER homeostasis and cause the accumulation of unfolded or misfolded proteins, i.e., a state of ER stress. If cells fail to cope with the stress, apoptosis is inevitable. Recently, ER stress has been reported to play an important role in the pathogenesis of neurological disorders such as cerebral ischemia and neurodegenerative diseases (1), but its involvement in the spinal cord diseases has not been fully discussed.

Objectives: To evaluate the effects of ER stress on spinal cord neurons more accurately, we conducted the present study using tunicamycin (Tm) as an ER stress inducer, and the organotypic slice culture system we have recently established (2). Moreover, studies with p53 inhibitor, pifithrin-α (PFT), were performed to investigate the role of p53 in the pathways leading to neuronal death.

Methods: The lumbar spinal cords were removed from neonatal Sprague-Dawley rats on day 7 and cut into 400-μm slices for organotypic slice cultures. After 10 days of incubation, slices were exposed to Tm at various concentrations with or without PFT. Anti-GRP78 and anti-β-actin antibodies were used as the primary antibodies in Western blot analysis. Immunohistochemistry and immunofluorescence studies were carried out with SMI-32 and anti-calretinin antibody for labeling motor neurons and dorsal horn interneurons, respectively. In addition, antibodies against p53 were used for double staining. The viability rate was obtained for statistical analysis.

Results: Tm was shown to induce ER stress by increased expression of an ER stress marker, GRP78. The viability rate of spinal cord neurons decreased in a dose-dependent manner by Tm treatment. Dorsal horn interneurons decreased significantly at low concentration of Tm at which motor neurons were not damaged. Immunofluorescence studies showed nuclear accumulation of p53 in the dorsal horns of Tm-treated spinal cord slices. PFT significantly increased the viability rate of dorsal horn interneurons, but not of motor neurons.

Discussion and conclusions: The findings of the present study suggest that dorsal horn interneurons are more vulnerable to Tm-induced neurotoxicity and that p53 plays an important role in the cell death pathway. On the other hand, p53 is not primarily involved in Tm-induced motor neuronal death, suggesting that the role of p53 may vary between different cell types. This difference may be a clue to the mechanism of the stress-response pathway, and may also contribute to the potential application of p53 inhibitors for the treatment of spinal cord diseases including ALS.

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P202 IMMUNOPROFILING OF NEURONS: MECHANISMS FOR THE UPTAKE OF MATERIAL BY NEURONS

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Introduction: We have documented the uptake of bulk material by neurons, including motor neurons (1). Neurons can uptake a wide range of debris including micro-organisms, cell debris and inert material. The mechanisms of these processes are not clear. Neurons are known to share certain antigens in common with cells of the immune system, Thy 1 related molecules being some of the best known, but systematic profiling has not previously been performed.

Materials and methods: Cortical neurons were obtained from adult C57B6 mice aged 6-10 weeks. Flow cytometric analysis was carried out using a range of antibodies to phagocyte and other surface markers. Antibody isotype controls were used, and positive cell standards were prepared from fresh blood or spleen. CD 90.2 (Thy 1.2) staining was used to identify neuronal populations, with gating for size and granularity. Data was analysed using a number of different statistical tests, including differences in median florescence between antibody and isotype control and the Kolmogorov-Smirnov (K-S) 'goodness of fit' test from which a Dmax value indicated separation of populations. Spinal cord lower motor, enteric and cortical neurons were profiled immunohistochemically using antibodies to a similar range of molecules as validation in human material.

Results: Of 15 markers studied by flow cytometry including CD49, CD51/61, TLR4 and CD14, five showed neuronal expression, including TLR4, CD29 and CD25 ($p < 0.05$) with markers such as CD14, CD11b, and CD40 being absent ($p > 0.05$). Immunohistochemical studies confirm a similar overlap with selected immune related molecules.

Discussion and conclusions: Neurons appear to express a specific, but restricted, set of surface receptors in common with phagocytes. It is likely that these surface

receptors contribute to functional aspects of neuronal phagocytosis, and may play a role in the uptake of a range of material from the external milieu, including material from glial cells.

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P203 TOWARDS A PERIPHERIN SIGNATURE FOR ALS

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Background: The formation of pathological inclusions, present in motor neurons of patients with amyotrophic lateral sclerosis (ALS), may be related to intra-neuronal intermediate filament (nIF) assembly properties. The nIF peripherin is associated with the generation of different protein isoforms that arise from alternative splicing or internal translation. We have previously observed that normal peripherin filament assembly is dependent on the co-expression of these isoforms, notably the constitutively expressed Per-58 and Per-45. We have recently established that peripherin isoforms associate together to form an intra-isoform ratio that is associated with the structural integrity of peripherin filament networks.

Objectives: We have observed tissue-specific differences in peripherin isoform expression. This pattern of expression is consistently visible on Western blots of different brain, spinal cord, and peripheral nervous tissue; we have termed this as a molecular signature for peripherin, with the profile of the signature changing depending on which neuronal regions are studied. Our objective is to substantiate these findings and to show that this signature can change in response to various types of stresses, such as stroke or sciatic nerve crush, and to compare between different mouse models of ALS.

Methods: Different regions of the brain and spinal cord were dissected and Triton X-100 soluble and insoluble fractions were prepared for analysis by Western blotting using peripherin antibodies. The mouse models used for this study were two lines of peripherin overexpressing mice (TPer and TPer;L-/-), mutant $SOD1^{G93A}$ mice, and mice that had undergone sciatic nerve crush or middle cerebral artery occlusion.

Results: Here, we have established that the relative ratio of peripherin isoform expression is tissue-specific and that this ratio is significantly altered between normal and disease-associated conditions. In normal mice, the level of isoform expression and the predominate peripherin species differed significantly depending on the neuronal region examined. Significant differences in the level of peripherin isoform expression and the relative isoform ratio were further observed between different disease-associated conditions in both the soluble and insoluble peripherin fractions, indicating multiple isoform-specific changes in peripherin processing in different mouse models. We have also observed other, as of yet, unidentified peripherin species that may further contribute to this signature.

Conclusions: Our results indicate that processing of peripherin to generate different protein isoforms, through alternative splicing or translation, can be characterized by isoform-specific patterns represented as a molecular signature for normal, regenerative, and pathological peripherin expression that may have relevance to our understanding as to how peripherin pathology is generated in ALS. Ultimately our goal is to define the peripherin molecular signature for ALS and to determine if this is consistent between different types of ALS or if there are specificities that might allow us to distinguish between different forms of the disease.

P204 PERIPHERIN ISOFORMS INTERACT WITH SIP30-ZWINT AND ALTER VESICLE TRAFFICKING

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Background: Peripherin is an intermediate filament (IF) protein expressed in motor neurons during development and in response to injury and disease. Peripherin is upregulated in motor neurons in familial and sporadic ALS and localized to inclusion bodies. Whether peripherin is protective or promotes cell death seems to depend upon the particular variant expressed through alternate splicing, in particular the neurotoxic Per61. Overexpression of peripherin in transgenic mice causes motor neuronal degeneration, and expression of Per61, but not non toxic Per58 or Per56, in cultured motor neurons is associated with aggregation into inclusions and cell death.

Objectives: This study was carried out to identify peripherin-interacting proteins that might contribute to pathogenesis of ALS.

Methods: The Yeast two-hybrid assay was used to screen a mouse brain cDNA library for peripherin-interacting partners. Interactions were confirmed by Far Western and coexpression in SW13- and in cultured motor neurons.

Results: Zwint was identified as a protein interacting specifically with peripherin but not the IF proteins NFL, NFM, or vimentin. Zwint-1 (a.k.a. SIP30) was previously identified as a binding partner of ZW10, a key component of the spindle check point and ER-Golgi trafficking in cell lines, and of SNAP25 in brain. In transfected SW13- cells, Zwint disrupted the peripherin IF network when co-expressed with Per58. Zwint prevented the aggregation of Per61 into punctuate inclusions, and both proteins were distributed diffusely or colocalized in small, abnormal

filamentous structures. Activation of PKC by phorbol ester shifted Zwint from a more diffuse cytoplasmic/nuclear distribution to vesicular structures. When present, Per58 colococalized with these structures, whereas Per61 and zwint colocalized in a dot-like pattern. In motor neurons of 3-week-old dissociated murine spinal cord cultures, which do not express peripherin, endogenous Zwint was concentrated in the perinuclear region, partially colocalized with SNAP25. When Per58 or Per61 were expressed, both Zwint and SNAP25 translocated to the cell processes and to the membrane with a difference between Per61 and Per58. A similar redistribution of Zwint was observed in motor neurons following activation of PKC. Finally, a homologue of Zwint was identified in human spinal cord, with high expression in motor neurons.

Discussion and conclusions: We have identified a specific peripherin binding partner that does not interact with other neurofilament proteins. The ability of Zwint to interact with SNAP25, suggests a normal role in SNARE-dependent vesicle trafficking. Our data show disruption of Zwint by peripherin, toxic and non-toxic forms having a different action on its localization, and by activation of PKC. In motor neurons, expression of peripherin and activation of PKC occur with injury, including ALS. Because both Zwint and SNAP25 translocate to the membrane under those conditions, we postulate that alteration of vesicular trafficking, exocytosis or neurotransmitter release could be the consequence. Further studies will be necessary to clarify the subcellular distribution of Zwint, how it is influenced by peripherin splice variants and signalling pathways, whether disruption is a common response to injury and whether helpful or harmful.

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P205 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ALTERNATIVELY SPLICED ISOFORM OF TDP-43

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Background: TDP-43 is a DNA and RNA binding protein which is involved in transcriptional repression and alternative splicing regulation. Recently, the mislocalization of TDP-43 from the nucleus to the cytoplasm to form inclusions has been found to be a pathological alteration in sALS and FTLD-u. Western blot analysis has also shown that insoluble moieties from diseased tissue contain phosphorylated full-length TDP-43, as well as low molecular weight bands which lack the N-terminus of TDP-43. According to the mouse EST database in Genbank, there are six additional alternative splicing isoforms of TDP-43. Four of them are involved in alternative splicing sites of the 3′-terminus of the pre-mRNA of TDP-43 and generate C-terminus truncated proteins lacking glycine-rich domains. Interestingly, the other two alternatively spliced ESTs consist of the splicing out of 91 nucleic acids in exon 2. The function of all TDP-43 isoforms is largely unknown.

Objective: The purpose of the following studies was to determine whether alternative splicing of TDP-43 contributes to the mislocalization of TDP-43 from the nucleus to the cytoplasm in ALS and whether this results in the appearance of low-molecular weight TDP-43 species on Western blots of SDS-polyacrylamide gels.

Methods: In the current study, we used RT-PCR to clone the full-length of the mouse N-terminal spliced isoform of TDP-43, a C-terminal short isoform of TDP-43 and full-length TDP-43. These isoforms were expressed in mammalian cell lines to examine the localization and phosphorylation changes resulting from TDP-43 isoform expression.

Results: In vitro expression studies demonstrated that the N-terminal spliced TDP-43 cDNA was transcribed from the third ATG and disrupted the nuclear localization signals located in the N-terminus. Immunostaining assays showed that this novel isoform of TDP-43 predominantly remained in the cytoplasm and had a propensity to form aggregates.

Conclusions: The present studies demonstrate that a novel alternatively spliced isoform of TDP-43 contributes to the mislocalization of TDP-43. Furthermore, from the human EST database, it is known that the human form of TDP-43 also has similar alternatively spliced ESTs. Whether the novel N-terminal splicing isoform contributes to the pathology of sALS and FTLD-u requires further examination.

P206 OVEREXPRESSION OF SMALL HEAT SHOCK PROTEIN HSPB8 IN CELLULAR MODELS OF AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL AND BULBAR MUSCULAR ATROPHY

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Small heat shock proteins (small HSPs) are molecular chaperones that protect cells against stress; they assist in the correct folding of denatured proteins preventing aggregation of misfolded proteins. Recently, mutations in two members of the small HSPs superfamily, HSPB1 (HSP27) and HSPB8 (HSP22), have been associated with peripheral neuropathies; HSPB8 was also observed in pathological neuronal tissue and has shown the ability to prevent *in vivo* formation of mutant huntingtin aggregates, suggesting an involvement of these proteins

in neurodegenerative diseases. The aim of this work was to investigate the role of HSPB8 in two different motoneuronal diseases: a familial form of amyotrophic lateral sclerosis (fALS) and spinal and bulbar muscular atrophy (SBMA). fALS is often associated to mutations in Superoxide Dismutase 1 (SOD1) gene, while SBMA is caused by polyglutamine tract expantion (polyQ) in the Androgen Receptor (AR), Although SOD1 and AR do not share structural or functional domains, their mutant forms are unstable and prone to form aggregates. In our studies, we used an immortalized motor neuronal cell line (NSC34) transfected with plasmids encoding for wild type and mutant forms of SOD1 (SOD1wt/G93A) or for mutant ARpolyQ (ARQ46). Both mutant proteins are characterized by: i) formation of intracellular aggregates, visible in immunofluorescence analysis, ii) the presence of PBS-insoluble materials, in filter retardation assay and iii) accumulation of YFPu (Yellow Fluorescent Protein with a degron signal for the proteasome system), indicating that proteasome activity is inhibited. Overexpression of HSPB8 in NSC34 trasfected with SOD1G93A and ARQ46 led to decrease in the levels of both mutant proteins (monomeric forms) and to reduction of either PBS-insoluble forms of mutant proteins, revealed by filter retardation assay, or SDS-resistant forms, that were present in Western blotting. Moreover, we observed a decrease in the levels of YFPu, that suggests a desaturation of the proteasome system. These results suggest that HSPB8 displays chaperone activity towards both mutated SOD1 and AR. Finally, the observation that HSPB8 displays its chaperone activity on mutant SOD1 even when the proteasome is impaired, suggests that this small chaperone could be a component of an alternative degradative pathway.

P207 ISOLATION AND PROTEOMIC CHARACTERIZATION OF MUTANT SOD1-CONTAINING INCLUSIONS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by loss of motor neurons resulting in progressive paralysis. Mutations in the gene encoding CuZn-superoxide dismutase (SOD1) have been associated with ALS and are found in ~5% of all cases. Multiple transgenic ALS murine models exist in which various mutant SOD1s are expressed. A characteristic pathological finding in both patients and murine models are inclusion bodies which in some cases display immunoreactivity towards SOD1.

Objectives: A possible toxic property of mutant SOD1 is interaction with proteins that become inactivated, depleted or erroneously activated. These proteins could co-aggregate with SOD1 and become part of cellular inclusion bodies. By isolation of very dense SOD1-containing particles from murine spinal cord tissue of terminal G127X and G85R ALS-models, we might have a system to characterize SOD1-containing inclusion bodies.

Method: Spinal cord tissue is homogenized gently (dounce glass pestle) in an isotonic buffer for preservation of organelle integrity. Nuclei are filtered away leaving mitochondria as the organelle with highest density. The homogenate is ultracentrifuged through a continuous Optiprep density gradient and organelle markers measured on collected fractions. A fraction of SOD1 is found in fractions with very high density. These are further evaluated by precipitation and 2-dimensional gel analysis/MALDI-TOF or by LC-MS/MS.

Results: Density gradient separations were performed on terminal G85R and G127X mice as well as on non transgenic littermates. Relevant fractions were precipitated by TCA and dissolved in urea buffer for proteomic analysis. From control animals only a few or no spots could be found on 2D gels. In G85R and G127X animals about 20–30 spots could be seen, most of which were overlapping between the models. These proteins were analyzed by MALDI-TOF mass spectrometry or by LC-MS/MS. So far about 10 proteins have been identified, some known before to be constituents of SOD1-inclusions such as GFAP and AB-crystallin.

Discussion: SOD1 containing inclusions are found in both ALS patients and terminal murine ALS-models. The composition of these has mainly been evaluated by immunohistochemical approaches which have the disadvantage of being limited by the availability of antibodies. By this method we are able to unveil previously unknown SOD1-aggregation partners as well as other proteins that might be trapped in these inclusions. To reveal such proteins might be valuable for the development of understanding the toxic mechanism of mutant SOD1s and the pathogenesis of ALS.

P208 THE RELATIONSHIP BETWEEN INCLUSION FORMATION AND CALCIUM DYSREGULATION IN MOTOR NEURON DISEASE

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Background: Dysfunction of the ubiquitin-proteasome system (UPS) and inclusion formation are hallmarks of ALS and experimental models (1,2). UPS impairment generally precedes formation of inclusions containing disease-causing mutant proteins (3). Motor neurons expressing ALS-associated mutant SOD1 ($SOD1^{mut}$) are protected from toxicity and formation of inclusions by treatments that reduce basal cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$); e.g., overexpression of the Ca^{2+} buffering protein calbindin

D-28K and blockade of Ca^{2+}-permeable ionotropic glutamate (AMPA) receptors (4).

Objective: We sought to determine any relationship between Ca^{2+} dysregulation, UPS dysfunction and formation of $SOD1^{mut}$ inclusions.

Methods: Plasmid encoding wild type or mutant SOD1 (G93A) fused to eGFP was expressed in motor neurons of dissociated spinal cord cultures by intranuclear microinjection at 4 weeks *in vitro*. Inclusion formation was monitored by fluorescence microscopy and $[Ca^{2+}]_c$ was monitored by ratiometric imaging using fura-2.

Results: Motor neurons expressing $SOD1^{mut}$-eGFP exhibited higher $[Ca^{2+}]_c$ than those expressing $SOD1^{wt}$-eGFP. $[Ca^{2+}]_c$ was further elevated in motor neurons that possessed visible $SOD1^{mut}$ inclusions compared to those that did not. Coexpression of calbindin D-28K reduced the percentage of motor neurons with $SOD1^{mut}$-eGFP inclusions, as well as the magnitude of $[Ca^{2+}]_c$ increase in those that did develop inclusions, evidence that buffering $[Ca^{2+}]_c$ can prevent formation of $SOD1^{mut}$ inclusions; however, calbindin D-28K failed to restore $[Ca^{2+}]_c$ to normal even in neurons lacking inclusions. Global upregulation of heat shock proteins (HSPs), achieved by expressing a constitutively active form of heat shock factor-1 (Hsf-1), reduced formation of inclusions, but did not reduce $[Ca^{2+}]_c$ in those motor neurons that did develop inclusions. Increase in $[Ca^{2+}]_c$ in motor neurons expressing $SOD1^{mut}$-eGFP was not a direct result of impaired proteasome function; the effect was not reproduced by treating cultures with epoxomicin for 7 days, which resulted in 60–70% reduction in proteasomal activity.

Summary and conclusions: Motor neuronal $[Ca^{2+}]_c$ increased with expression of $SOD1^{mut}$ and a secondary rise was associated with formation of inclusions. Neither increase appeared due to a direct result of proteasomal inhibition. Rather, the ability of calbindin D-28K to reduce both inclusion formation and the magnitude of $[Ca^{2+}]_c$ rise in motor neurons with inclusions suggests that a critical increase in $[Ca^{2+}]_c$ promotes aggregation of $SOD1^{mut}$. Formation of inclusions and the related secondary rise in $[Ca^{2+}]_c$ also was prevented by upregulating HSPs, suggesting that formation of inclusions dysregulates Ca^{2+}. This apparent dichotomy would be resolved if the secondary increase in $[Ca^{2+}]_c$ were temporally correlated with, but not responsible for, inclusions or if HSPs protect through mechanisms upstream of Ca^{2+} in addition to chaperoning $SOD1^{mut}$. Preliminary studies implicate uptake of Ca^{2+} by mitochondria and possibly ER in triggering the secondary rise in $[Ca^{2+}]_c$ and formation of inclusions.

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P209 THE α-SUBUNIT (CD11B) OF COMPLEMENT RECEPTOR TYPE 3 MODULATES MICROGLIAL FUNCTION INVOLVED IN MAP KINASE SIGNALLING PATHWAY

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Background: Microglia are considered as the resident immune cells of the central nervous system (CNS). They play important roles in neurodegenerative diseases by producing several proinflammatory cytokines and nitric oxide (NO). An upregulation in the complement receptor type 3, a complex of α-subunit (CD11b) and β-subunit (CD18), in the impaired brain regions has been found in almost all cases of end stage of various neurodegenerative diseases in autopsy. It has also been demonstrated to be one of the earliest markers up-regulated in activated microglia, suggesting that it may play a role in regulation of microglial activities. We have previously reported that a downregulation in CD11b expression and the formation of neuronal protein aggregation could be noted in the spinal cord during early development of neurofilament light subunit knockout (NFL$-/-$) mice. However, when the mice grew older, glial reaction and further neuronal loss could be observed. Other reports have indicated that CD18 may not be responsible for fulfilling some microglial functions, such as NO generation.

Objectives: In order to understand the meaning of the downregulation of CD11b expression in the earlier age of the NFL$-/-$ mice, we have used the transient siRNA transfection approach to specifically knockdown CD11b gene expression in BV-2 microglial cells and explored the role of CD11b in microglial activities and the cellular signalling mechanism induced by LPS.

Methods: Fluorescent-labelled control was used to monitor transfection efficiency. The BV-2 cells transfected with scrambled siRNA at 48 and 72 hours served as negative control. Microglial intracellular NO generation was monitored by using DAF-2DA (4, 5-diaminofluorescein diacetate) and phagocytosis by latex beads. The action of MAP kinase pathway was examined by Western blot. The conditioned medium collected from 24-hour LPS-stimulated CD11b-knockdown or control cells was applied to NSC-34 cells for 24 and 48 hours to monitor the cytotoxicity.

Results: In an optimized condition, nearly all the cells were labelled with Cy3. Real-Time PCR showed that there was around 88% and 82% decrease in CD11b mRNA expression in BV-2 cells transfected with CD11b siRNA at 48 and 72 hours post-transfection respectively. Upon LPS stimulation, remarkable increases of NO generation and beads intake in normal and negative control BV-2 cells were significantly reduced in CD11b siRNA cells. In addition, marked phosphorylation of ERK, JNK and P38 proteins and increases in IL-1β mRNA observed in normal

BV-2 and the negative control cells were also significantly inhibited in CD11b knockdown cells. The conditioned medium from LPS-simulated CD11b-knockdown BV-2 cells was found to be obviously less toxic to NSC-34 neurons than that from the controls.

Conclusion: The inhibition of CD11b by siRNA may reduce microglial activation via MAP kinase pathway. The downregulation in CD11b expression may ameliorate the inflammation in neurodegenerative diseases, suggesting a possible inhibitory mechanism for early neuropathogenesis in certain neurodegenerative diseases.

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P210 A TISSUE-ENGINEERED MODEL TO STUDY AXONAL MIGRATION AND MYELINATION OF MOTOR NEURONS IN VITRO

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Background: Amyotrophic lateral sclerosis and other motor neuron diseases are very complex as their development and progression involve many cellular processes and many cell types. To better understand and study motor neurons, in defined conditions and in a physiological environment that reflect the *in vivo* reality, a three-dimensional tissue-engineered *in vitro* model was developed by our team.

Objectives: Our aim was to study motor neuron biology in this reconstructed tissue in order to better characterize it and to further use it as a physiological *in vitro* model of degenerative diseases affecting motor neurons.

Methods: Purified mouse spinal cord motor neurons (1) were seeded on a collagen and chitosan sponge biomaterial populated with Schwann cells and/or fibroblasts (2). This fibroblast-populated sponge mimicked the connective tissue through which motor axons have to elongate *in vivo*. Tissues with motor neurons were cultured for 14, 21 and 28 days before analysis were performed. Motor neuron neurite elongation was assessed by immunofluorescence followed by microscopic observations and quantification. Myelination was assessed by immunofluorescence and transmission electronic microscopy.

Results: Our reconstructed tissue revealed itself to be a very favourable environment for the survival, neurite elongation and maturation of motor neurons. This was visualized by immunofluorescent staining of the different neurofilaments. The presence of Schwann cells in the tissue allowed myelination of the motor neuron axons. Myelin basic protein expression by Schwann cells as well as electronic microscopy observation of myelin sheaths demonstrated the abundant myelination that occurred in the tissue. Moreover, Schwann cells promoted the elongation of more motor neuron neurites in the tissue.

Discussion: This indicated that our tissue is a representative model of the *in vivo* physiology of motor neurons. All these characteristics make our reconstructed tissue well-suited for *in vitro* motor neuron studies that require a physiological three-dimensional environment.

Conclusion: Comprehension of amyotrophic lateral sclerosis and other motor neuron diseases pathogenesis will surely be greatly improved by the use of this highly promising model.

Acknowledgments: The Muscular Dystrophy Association and the Fonds de la Recherche en Santé du Québec.

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P211 ASTROCYTE-SPINAL NEURON CO-CULTURE AS *IN VITRO* MODEL OF ALS TO EXAMINE INTER AND INTRACELLULAR MECHANISMS ASSOCIATED TO MOTOR NEURON DEGENERATION

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Background: The study of *in vivo* animal models of ALS has provided clues about the mechanisms underlying motoneuron death. However this approach is often challenging because of the variety of the cell types and pathways involved. Thus, the use of reliable *in vitro* models can help in deciphering the factors that affect motoneurons. Recent work has pointed out the role of astrocytes in this phenomenon, showing their toxic influence on motoneurons when they express SOD1 mutants. However, the molecules involved have not yet been identified.

Objectives: We set up astrocyte-spinal neuron co-cultures to obtain a simplified model of motoneuron death and investigate the nature of the unknown factors involved in the harmful astrocyte-motorneuron cross-talk and the role of intracellular signaling .

Methods: Primary astrocyte-spinal neuron co-colture was prepared where SOD1-G93A was only expressed in spinal neurons, only in astrocytes or both. The motoneuron survival was evaluated by cell count. The analysis of the supernatant was performed by proteomic approach.

Results: We observed that isolated astrocytes carrying SOD1-G93A were unable to release detectable levels of proinflammatory cytokines while high levels of mutant SOD1 and other proteins, still under identification, were found in the cell medium. In astrocyte-spinal neuron co-culture a significant and remarkable motor neuron death was observed when mutant SOD1 was expressed in astrocytes or motor neurons or in both cell types suggesting that the presence of mutant SOD1, independently of the cell type in which it was expressed, is sufficient to trigger motoneuron degeneration. However, when wild type neurons were grown in the medium obtained from the different combinations of mutant SOD1 expressing co-coltures (6 DIV), no motoneuron death was observed suggesting that either transient soluble factors or a direct contact with astrocytes may be involved. Further studies are ongoing to address this issue.

Conclusion: We obtained an *in vitro* model of co-culture that reproduces motoneuronal death when SOD1-G93A is expressed only in astrocytes, only in neurons or both.

P212 ALS-LIKE AXONAL PATHOLOGY IN CULTURED SPINAL MOTOR NEURONS

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ALS is characterized pathologically by the presence of abnormal proteinaceous inclusions, and in particular neurofilamentous spheroids within the axons of spinal motor neurons. However, important links between this pathology and potential causative factors have not been clearly established. We have investigated the role of excitotoxicity, oxidative stress and glial support in the induction of pathological neurofilament changes in spinal motor and cortical neurons maintained in long-term culture. Spinal motor and cortical neurons were derived from embryonic rodents, and seeded onto a feeder-layer of mixed cortical glial cells or purified astrocytes, derived from P3 rodents. Spinal neurons were concentrated on an optiprep™ density gradient, yielding a subset of motor neurons immunopositive for SMI32, an antibody to de-phosphorylated neurofilaments. Cortical neurons were seeded directly onto feeder layers. Cultures were maintained for up to 21 days, followed by paraformaldehyde-fixation and immunolabelling for cytoskeletal proteins. To investigate cytoskeletal changes following excitotoxicity, neurons were treated with 25, 50, 100 μM kainic acid (KA), NMDA or vehicle, plus or minus CNQX and MK801, for up to 72 hours. To investigate the role of glial support cells in the induction of cytoskeletal pathology, neurons were seeded onto mixed glial cells. Neurons were also grown on glia derived from G93A mutant SOD1 (mSOD1) neonates. Oxidative stress was induced *in vitro* by growing cultures in antioxidant-free media.

Spinal motor neurons grown on mixed glial feeder layers developed proximal axonal swellings, resembling axonal spheroids of ALS, which were immunopositive for both phosphorylated and de-phosphorylated neurofilament triplet subunits. Swellings were present in neurons grown in both normal and antioxidant free media, and on mSOD1 feeder layers. In contrast, excitotoxicity resulted in a more distally directed axonopathy, consisting of large swollen abnormal neurites accumulating both phosphorylated and de-phosphorylated neurofilament triplet subunits. This axonopathic effect of excitotoxicity was present in spinal motor but not cortical neurons in culture, and was mediated predominantly through non-NMDA receptors within 6 hours of exposure to excitotoxin. These studies indicate that the axons of cultured spinal motor neurons, but not cortical neurons, were particularly susceptible to axonal swelling and neurofilamentous accumulation induced both by the surrounding glial environment and excitotoxicity. However, excitotoxicity was closely linked to a distal axonopathy whereas a proximal axonopathy was associated with the effects of growing spinal neurons on a mixed glial feeder layer. These new cell culture models will act as a platform for further investigation into the mechanisms involved in the loss of axonal function and may be helpful for examining therapeutic approaches in ALS.

P213 TROPHIC REQUIREMENTS FOR LONG-TERM SURVIVAL OF MATURE AVIAN MOTONEURONS IN ORGANOTYPIC SPINAL CORD CULTURES

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We have developed an organotypic spinal cord culture technique in which trophic requirements for long-term survival of mature motoneurons were studied (1). Spinal cord slices were obtained from chick embryos on E16. At this age, chick embryo motoneurons are essentially target independent for survival and maturational processes characteristic of the postnatal period in rodents (e. g., regression of muscle polyneuronal innervation), have already occurred resulting in a relatively mature stabilized neuromuscular synaptic pattern. Because motoneuron degeneration and cell death appear to differ between infantile and adult motoneuron diseases, this avian model may be a valuable tool to develop experimental approaches that more closely mimic adult motoneuron pathology than currently available rodent models.

Slices were maintained for up to 28 days *in vitro* (DIV) in a basal medium. Under these conditions, most motoneurons died. To promote motoneuron survival, 14 different trophic factors were assayed. Of these 14, GDNF and VEGF were the most effective. GDNF was able to promote motoneuron survival for at least 28 DIV. In absence of neurotrophic factor support, K^+ depolarization or caspase inhibition prevented motoneuron death. The effect of K^+ was mediated by Ca^{2+} influx through

voltage-gated channels, since it could be inhibited by different L-type Ca^{2+} channel antagonists. However, after high K^+ or caspase inhibition, motoneurons although alive developed degenerative-like changes. Agents that elevate cAMP levels, such as forskolin, IBMX or pcPT-cAMP, promoted the survival of a proportion of motoneurons for at least 7 DIV. Examination of dying motoneurons revealed that in addition to cells exhibiting a caspase-3-dependent apoptotic pattern, some motoneurons died by a caspase-3-independent mechanism and displayed autophagic vacuoles, an extremely convoluted nucleus and a close association with microglia. This organotypic spinal cord slice culture may provide a convenient model for testing conditions that promote survival of mature-like motoneurons that are affected in late-onset motoneuron disease such as amyotrophic lateral sclerosis.

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P214 EVIDENCE FOR THE DELETERIOUS EFFECT OF K40I ANGIOGENIN MUTATION IN MOTONEURON DEGENERATION

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Background: Angiogenin (ANG), a hypoxia-inducible ribonuclease mainly associated with angiogenesis, is an attractive candidate to play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS). Seven missense mutations in the gene coding for ANG, five of them affecting functionally important residues, have been found in several patients with sporadic and familial forms of ALS (1). Moreover, ANG can also regulate the activity of growth factors like VEGF, which in turn has been shown to act as a neurotrophic factor for motoneurons.

Objectives: To study the functional effect *in vitro* of K40I ANG mutation. This point mutation, found in several patients, affects a highly conserved and critically important catalytic residue, and therefore it is predicted to induce a significant loss of protein activity.

Methods: We cloned the human ANG sequence and introduced the K40I mutation by site directed mutagenesis. Transient transfection and viability studies were then performed in the motoneuron-like murine NSC34 cell line, exposing cultures to glutamate toxicity or tunicamycin-induced ER stress. siRNA experiments to knock down ANG were also carried out in NSC34 cells exposed to serum deprivation and in primary motoneuron cultures exposed to AMPA.

Results: Here we report that overexpression of ANG induces a significant increase in NSC34 cell survival, both in glutamate toxicity and under ER stress. This neuroprotection is not observed when cells overexpress the K40I mutant form. Suppression of ANG also exacerbates serum deprivation toxicity in NSC34 cells and AMPA-mediated excitotoxicity in motoneurons.

Conclusion: Our data supports the neuroprotective role of ANG in vitro and the involvement of the K40I ANG mutation in the motoneuron degeneration found in ALS patients.

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P215 EFFECTS OF LUTIMAX™, AN ANTIOXIDANT COCKTAIL SUPPLEMENT, ON MOTOR NEURON SURVIVAL *IN VITRO*

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Background: The multiple pathways involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) provide multiple targets for drug therapy. The approach of using combinations of drugs and nutriceuticals has been studied using the SOD1 mouse model with success. These studies, however, have not translated with the same effect when tested in clinical trials. Our laboratory has developed an *in vitro* model system to study motor neuron cell death using a panel of chemical inducers with different mechanisms of action. These compounds mimic some of the pathways shown to be involved in cell death in patients with ALS.

Objective: The current study was designed to examine the neuroprotective ability of a dietary supplement, Lutimax ™, in our cell death model system. Lutimax ™ contains a combination of antioxidants including vitamins (C, D and E), calcium carbonate, luteolin and rutin with inactive components. Anecdotal evidence indicates that this supplement may have benefit in patients with ALS but no controlled experiments examining its effects have been performed.

Methods: Using differentiated NSC34 cells, the toxicity of Lutimax ™ alone was examined in a dose-response study. Cell death was assessed after a 24hr exposure. The concentrations were based on the amount of the two most active components, luteolin and rutin, present in the supplement. The doses ranged from 0 mg/ml to 10 mg/ml. We then examined whether Lutimax ™ could improve cell survival of differentiated NSC34 cells following exposure to 4 cell death inducing agents: staurosporine, thapsigargin, carbonyl cyanide cholorophenyl hydrazone (CCCP) and hydrogen peroxide. NSC34 cells were pretreated for 2hrs with 1 mg/ml Lutimax ™ or solvent control (0.1% DMSO) and then exposed for 24hr to each

of the cell death inducers. Cell death was determined using nuclear staining and morphology with Hoechst 33342 and propidium iodide.

Results: We determined that doses of 0.1 and 1 mg/ml Lutimax ™ were non-toxic to NSC34 cells using a nuclear morphology staining assay. The 10 mg/ml dose reduced cell viability by 80% ($p \leqslant 0.05$). We then examined whether pre-treatment with Lutimax ™ reduced cell death in cultures exposed to various death inducers. NSC34 cells treated pre-treated 2hr with Lutimax ™ and then exposed to staurosporine and hydrogen peroxide for 24hr had greater survival then with inducer alone (reduced by 11.75% and 8.17%, respectively; $p \leqslant 0.05$). No significant improvement in cell survival was observed with CCCP or thapsigargin, although there was a trend towards improvement with thapsigargin. Additional assays to confirm these results and to examine the active agents as 2 or 3 pure compounds in combination are underway.

Conclusions: These data suggest that the components of Lutimax ™ do improve motor neuron survival and provide another candidate compound for combining with other potential therapies.

P216 SMN-DEPLETED NSC-34 NEURONS SHOW REGULATION OF GENES INVOLVED IN NERVOUS SYSTEM DEVELOPMENT, APOPTOSIS, AND MITOCHONDRIAL FUNCTION

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Background: Low survival motor neuron (SMN) protein concentration is responsible for lower motor neuron death in spinal muscular atrophy (SMA). SMN protein is a component of RNA processing complexes, as well as axonal cones. A C-terminal portion of the protein appears to be important for motor neuron integrity and axonal development. However, it is unclear which molecular pathways are affected in SMN loss.

Objectives: To determine the cascade of gene regulation associated with SMN loss in neuronal cell cultures.

Methods: We used a mouse neuronal cell line (NSC-34), which models key features of developing motor neurons. The SMN expression in NSC-34 cells was lowered using small interfering RNA (siRNA) transfection. Total mRNA was extracted from cells 48 and 72 h after transfection and compared to controls using microarray analysis with subsequent confirmation of target gene regulation by quantitative real-time RT-PCR. Differential expression profiling was performed using a full genome mouse oligonucleotide array (Agilent). These data were further analyzed using DAVID gene ontology comparison software to highlight enriched biological themes. The MTT assay and ATP production were measured at 48 and 72 h to assess mitochondrial function.

Results: SMN protein level was decreased by 65–70% of controls 72–96 h after transfection of siRNA. In addition to the expected decrease in SMN mRNA levels, we identified significant changes in expression of genes involved in apoptosis, cell division and fibroblast growth factor 2 (FGF-2), previously reported to be associated with SMN regulation. Biological themes that were significantly over-represented in the list of genes included cell division and transcriptional regulation as well as programmed cell death. Differential expression analysis between 48 and 72 h highlighted biphasic changes in expression of genes involved in nervous system development, such as Mtap1b and FGF-2, and apoptosis, such as Casp3. Further cluster analysis indicated that ''mitochondria'' was the most significantly enriched theme among significantly regulated genes measured at 48 h. Cellular ATP was decreased relative to control when measured at 48h and decreased further at 72 h.

Discussion and conclusions: Carefully designed gene expression profile experiments combined with appropriate convergent assays can be used to determine cascades of gene regulation. The application of this approach to a model of SMA has confirmed the regulation of genes previously implicated in the loss of lower motor neurons. These data further support an apoptotic cell death, as indicated by both the gene regulation associated with apoptotic markers and the primacy of mitochondrial mechanisms as indicated by mitochondrial gene regulation and decreased cellular ATP levels. This report confirms the utility of *in vitro* neuronal cell culture systems in dissecting the mechanisms involved in SMA pathogenesis and suggests modalities for its potential rescue.

P217 ARGININE METHYLATION OF LAMIN IS INVOLVED IN THE FUSION OF MYOBLASTS

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Background: Fusion of myoblasts is important in the development (1,2) of myotubes. We have studied morphological characters of myoblast fusion. Recently we tried to detect 'key fusion regulator proteins'. Western blotting and immunofluoresence studies showed increased activity of the protein arginine methyltransferase activity (PRMT) during the myoblast fusion *in vitro*(3).

Objectives: This study was designed to find the methylated proteins by PRMT during the rat myoblast fusion and to identify the possible site(s) of arginine methylation.

Methods: Myoblasts isolated from hindlimbs of E21 Sprague-Dawley rats were cultured (4–6). Total proteins were extracted at three different developmental points; before fusion, early fusion, and after fusion of myoblasts. After two-dimensional electrophoresis, immunoblotting against asymmetric dimethyl arginine 24 and symmetric dimethyl arginine 10 antibodies were performed. Methylated proteins were identified by standard proteomic techniques; image analysis, MALDI-TOF (Bruker Autoflex, German) mass spectrometry, and the database search using Mascot. Among the methylated proteins during the myoblast fusion, lamin was isolated by immunoprecipitation technique. Methylation of the isolated lamin was confirmed by Western blot analysis. The sequences of isolated lamin were investigated by LC-MALDI-TOFTOF-MS (PE-Sciex, Ontario, Canada) mass spectrometry(7,8).

Results: By immunoproteomic approach, we found 10 proteins were methylated during the myoblast fusion. Among these PRMT's possible substrate proteins, the sequence of the lamin was identified.

Discussion and conclusions: Present study suggests that PRMT methylates several proteins including lamin A/C during the myoblast fusion. Lamin A/C may act as 'key fusion regulator proteins' playing essential roles in skeletal muscle generation. Also lamin is known to be deeply related with the muscular dystrophy(9,10). We are searching for the precise site(s) of arginine methylation.

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THEME 9 GENETICS

P218 SPINAL MUSCULAR ATROPHY, CLINICAL AND GENETIC PATTERN

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Background: A country with a population of about two million. The study was conducted in a tertiary care University Hospital. Children with neuromuscular disorders are referred to this centre.

Objectives: To determine the pattern of clinical and genetic profile of Spinal Muscular Atrophy in this country.

Methods: A retrospective study from 1992 to December 2001 followed by a clinical and genetic prospective study from January 2002. Data for the retrospective study was collected from neurophysiology records, from in- and outpatient files. The genetic study was funded by the university. The patients were prospectively worked up for the SMN gene deletion. The patients were classified as having type I, II and III SMA based on their clinical features as per the International Consortium Classification. Family history was recorded in all cases.

Results: Ninety-five patients were seen. Sixty-two cases were of SMA type I (65.3%), 16 of type II and 17 of type III. In SMA I, onset younger than 1 month old was seen in 42 cases, 12 had onset between 1 to 3 months and 8 cases in 3 to 6 months. Positive family history in siblings was found in 48 cases. There was history of SMA in other relatives in 13 cases. Consanguinity was in 45 (49%), negative in 20 and unknown in 27. Seventy per cent of SMA cases showed Survival Motor Neuron gene deletion. Eighty three percent of SMA I had SMN deletion.

Conclusions: The incidence is about one per six thousand live births (about forty thousand births per year). A new case is seen every two to three months. Spinal muscular atrophy type I forms 65% of cases. The deletion was 83% in spinal muscular atrophy type I. This center could participate in treatment trials.

P219 CONTRIBUTION OF REEP1 GENE MUTATIONS TO HEREDITARY SPASTIC PARAPARESIS (HSP)

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Background: HSP is a heterogeneous group of inherited disorders causing progressive spasticity and weakness of the lower limbs. So far 15 genes of the published 35 SPG loci have been identified. *Spastin* (*SPG4)* and *atlastin (SPG3A)* account for about 50-60% of AD HSP in total. Mutations in the receptor expression enhancing protein 1 (*REEP1)* were recently identified as a cause of HSP. A previous study of *REEP1* on a cohort of HSP patients revealed a mutation frequency of 7%. We hypothesised that *REEP1* mutation would be an important and a common cause of HSP in our cohort of HSP pedigrees. *REEP1* is predicted to be a nuclear encoded mitochondrial protein which led us to hypothesise that mutation in *REEP1* would be associated with mitochondrial dysfunction.

Objectives: 1) To establish the frequency of *REEP1* mutation as a cause for AD HSP. 2) To describe the clinical phenotypes of the patients with identified *REEP1* mutation(s).

Methods: 130 familial and 82 sporadic HSP patients, negative for *spastin (SPG4)* mutations, were screened. Exon –intron boundaries of 7 exons including 600 bp of 3′ UTR of *REEP1* were amplified using intronic primers and PCR products were directly sequenced.

Results: Two novel mutations were identified. The first in exon 5, c.339 C > T, p.113 stop codon TGA resulted in a premature truncation of the *REEP1*. The mutation segregated with the affected and not in unaffected family members. The phenotype in this family was an early onset spastic paraparesis complicated by the presence of distal amyotrophy. Previous muscle biopsy studies have shown that the index case had mitochondrial respiratory complex IV deficiency. The second novel mutation c.606+567 G > A was detected in the 3′UTR. The index case in this pedigree had a young age of onset (3 years) of AD HSP. The phenotype was complicated by the presence of dystonic type movement disorder. A previously described mutation at c.606 +43 G > T in the 3′UTR which alters an miRNA site, was also detected in a third case with AD HSP. A pure form of AD HSP was observed in the third case. These mutations were not detected in 262 control chromosomes, assessed by restriction enzyme digest. A

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further 82 index cases with sporadic spastic paraparesis were screened, but mutations were not detected.

Conclusions: Our data argue that the frequency of *REEP1* mutations is 2–3% (3/130) in AD HSP patients in the UK. It is worth screening this gene in patients with AD HSP who are negative for spastin screening. However, we found no evidence of REEP1 mutations in patients with sporadic spastic paraparesis. Early age of onset, distal amyotrophy, as well as muscle biopsy evidence of mitochondrial respiratory chain deficiency may be noteworthy clinical features of this subtype of HSP.

P220 A FAMILY WITH AN AUTOSOMAL DOMINANT PHENOTYPE CONSISTENT WITH PRIMARY LATERAL SCLEROSIS

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Background: Primary lateral sclerosis (PLS) is a disorder characterized by spinal and bulbar spasticity due to degeneration of the upper motor neuron. We describe here a French-Canadian family with progressive involvement of the upper motor neuron that mimics closely the features of PLS.

Objectives: We present detailed phenotypic and genotypic data of a French-Canadian family with a new autosomal dominant disease characterized by progressive upper motor neuron involvement.

Methods: A total of 8 affected and 10 unaffected family members underwent a thorough neurological examination, and were sampled. Most patients also underwent electrophysiological studies and imaging.

Results: This new disease entity can be viewed as a late onset disease that begins asymmetrically in the lower extremities by weakness and spasticity. The average age of onset is 48.4 years (range 30–60 years). Affected individuals will develop progressive spastic paraparesis, followed closely by involvement of the upper extremities with again weakness and spasticity. Then, the bulbar level will be involved with the emergence of a spastic dysarthria and eventually dysphagia. Electrophysiological testing shows no signs of lower motor neuron involvement, while imaging of the neuraxis may show mild spinal cord atrophy. The proband was tested for a wide range of acquired and inherited diseases which all came back negative (B12 deficiency, adrenomyeloneuropathy, vitamin E deficiency, SCA 1-2-3-6-7-8-10-14-17). In addition, we performed linkage analysis to exclude ALS1, SPG3A, and SPG4.

Conclusions: With the exception of the inheritable component of our newly described entity, the clinical phenotype conforms readily with that of PLS, even according to strict criterias. We have excluded the most obvious acquired and inherited forms that could have confounded our evaluation. A whole genome analysis is under way to localize this entity to a defined chromosomal region.

P221 TOWARDS IDENTIFICATION OF A NOVEL GENE FOR PROGRESSIVE MOTOR NEURON DEGENERATION ON CHROMOSOME 7

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Background: The motor neuron diseases (MND) are a group of related neurodegenerative diseases that cause the selective progressive death of motor neurons. These diseases range from slowly progressive forms including hereditary motor neuropathy (HMN), to the rapidly progressive disorder amyotrophic lateral sclerosis (ALS). It is increasingly being recognized that non-ALS motor neuron diseases show clinical and pathological overlap with forms of ALS and some forms share the same genetic basis, i.e. the same gene causes rapid or slowly progressive disease. As such, we anticipate that their distinction may, at least in part, be artificial.

Objectives: Our aim is to use molecular genetic approaches to identify new disease genes for familial ALS and HMN and establish whether these are more broadly involved in familial and sporadic MND/ALS, as pathogenic or susceptibility genes.

Methods: We have collected large cohorts of ALS (>160) and HMN (>50) families. In one large HMN family, we previously localised a novel HMN locus to chromosome 7q34–q36. Fine genetic mapping was performed in this family to identify the minimal recombination defined candidate interval. Linked genetic markers were genotyped in other large HMN families using standard dye-labelled techniques. Expression profiles were established for all genes within the chromosome 7q34–q36 interval using data available from the genome annotation projects (UCSC genome browser and Ensembl). Mutation analysis of candidate genes in the candidate region was performed using direct DNA sequencing.

Results: Candidate genes from the chromosome 7q34–q36 candidate interval were identified based upon their known or inferred function, or expression in affected tissues. *CDK5* was selected for immediate mutation analysis based upon its known association with an ALS like phenotype in mice, however no mutations were identified. A screen of further selected candidate genes is underway. An investigation of the prevalence of this disease locus among other non-ALS MND families is also underway.

Discussion: We have identified a locus for non-ALS MND on chromosome 7q34–q36. Work is underway to clone the gene in question. Once identified, we will

investigate large cohorts of non-ALS MND families, FALS, and sporadic ALS cohorts to establish whether this gene, like other MND genes, is involved in the slowly progressive disorders of motor neurons (HMN) and rapidly progressive disease (ALS), as a pathogenic or susceptibility gene.

P222 INVESTIGATION OF THE ROLE OF SMN1 AND SMN2 HAPLOINSUFFICIENCY AS A RISK FACTOR FOR HIRAYAMA'S DISEASE: CLINICAL, NEUROPHYSIOLOGICAL AND GENETIC CHARACTERISTICS IN A SPANISH SERIES OF 13 PATIENTS

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Background: Hirayama's disease (HirD), also known as monomelic amyotrophy (OMIM 602440) is an unusual form of adult-onset lower motor neuron disorder characterized by weakness and wasting confined to the hand and forearm (oblique amyotrophy) at young ages, mainly among males, with no fasciculations, and exacerbated by exposure to cold (cold paresis), with no sensory or pyramidal tract involvement. The etiopathogeny of the disease is a matter of debate. Although biomechanical factors during neck flexion have been proposed as the main cause of this disease, some underlying genetic factors are thought to play a role in the disease's etiopathogenesis.

Objectives: To test whether *SMN1* and *SMN2* copy numbers are susceptibility factors for HirD, and whether they are influencing factors in the severity of the resulting phenotype.

Methods: Clinical, EMG, MRI, and qualitative and quantitative *SMN1*/*SMN2* gene studies in two university-affiliated hospitals. The study included patients with HirD diagnosed according to clinical, electromyographic and MRI criteria, and involved neurological and neurophysiological examinations, cervical spinal MRI and qualitative and quantitative genetic studies for the *SMN1* and *SMN2* genes.

Results: Thirteen unrelated patients (11 men, 2 women) with HirD participated in the study. Unilateral arm amyotrophy was present in ten patients. Signs of sympathetic nervous system were observed in ten patients. Unilateral flattening or atrophy of the cervical cord was detected in four patients. In four patients with unilateral clinical involvement, EMG showed neurogenic changes on both the clinically affected and unaffected sides. No patient showed a homozygous deletion of either the *SMN1* or *SMN2* gene. No differences were found when comparing the *SMN1* and *SMN2* copy number distributions of the normal population and HirD patients. There was no correlation between the number of copies and the severity of the phenotype.

Conclusions: The *SMN* genotypes do not increase the susceptibility to this disorder. No correlation was found when comparing the number of copies of the *SMN1* and *SMN2* genes and the resulting phenotype in Hirayama's disease patients. The predominant occurrence in male patients, mostly of Asian extraction, and the description of familial cases, suggests that underlying genetic factors other than the *SMN* genes may be involved. According to these results, further genetic studies are necessary.

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P223 PROGRESSIVE MUSCLE ATROPHY WITH HYPOKALEMIC PERIODIC PARALYSIS AND CALCIUM CHANNEL MUTATION

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Background: Motor neuron degeneration in conjunction with hypokalemic periodic paralysis (HypoPP) has been recognized as distinct from HypoPP-related myopathy. Several studies have described familial cases of HypoPP that had developed a disabling motor neuron disorder resembling spinal muscle atrophy or progressive muscle atrophy (PMA). At the time of these reports, the molecular basis of HypoPP was unknown.

Patients and methods: We report a patient with familial HypoPP and a fatal course of PMA. In the studied family HypoPP arose from a mutation in the calcium channel gene *CACNA1S* encoding p.R528H which has been related to HypoPP before. The index patient demonstrated a clinical syndrome of HypoPP in conjunction with PMA. Postmortem studies showed cystatin-C- positive neuronal inclusions which are common morphological features of PMA and the classic form of ALS.

Discussion: Given the rarity of HypoPP and PMA, a chance association of the *CACNA1S* mutation and this phenotype was unlikely. A single case of HypoPP with PMA within the genetic trait of *CACNA1S*-linked HypoPP does not permit the conclusion that the PMA phenotype is caused by the calcium channel mutation. Nevertheless, the *CACNA1S* gene may represent a previously unknown genetic risk factor of PMA. It is well

conceivable though that genetic variants of the *CACNA1S* gene may contribute to a common pathway in HypoPP, vacuolar myopathy and motor neuron disease.

P224 A NOVEL FORM OF AUTOSOMAL RECESSIVE JUVENILE AMYOTROPHIC LATERAL SCLEROSIS MAPS WITH EQUAL PROBABILITY TO TWO AUTOSOMAL LOCI

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by progressive deterioration of both upper (UMN) and lower motor neurons (LMN). Five to ten percent of ALS cases are familial. Approximately 25% of familial cases are autosomal dominant due to mutations in the *SOD1* gene, which may have a juvenile onset. Juvenile recessive ALS (JRALS) may present with a varying degree of prominent upper motor neuron features. Three types of JRALS have been described including: type 1 (predominantly LMN); type 2 (a combination of LMN and UMN, with bulbar sparing); and type 3 (with prominent UMN involvement). Type 1 has been linked to the *ALS5* locus on chromosome 15q, and type 3 is most commonly due to mutations in the *ALS2* locus on chromosome 2q33.

Objectives: We have identified an apparently novel juvenile recessive motor neuron syndrome, affecting upper and lower motor neurons in both the limb and bulbar distribution. We present the clinical and electrophysiological features in the affected four of six offspring from a consanguineous (first cousin) marriage. We sought to map and identify the gene responsible for this form of motor neuron disease.

Methods: An initial genetic screen was performed to exclude linkage to known genetic loci associated with ALS, as well as two neuromuscular disorders with potentially overlapping phenotypes, Charcot-Marie-Tooth (CMT) and hereditary spastic paraplegia (HSP). Subsequently, we performed a full genome scan for regions of shared homozygosity, using microsatellite markers.

Results: Genotyping studies revealed a lack of linkage to previously reported loci associated with familial ALS, HSP or CMT. Our linkage studies identified two autosomal loci with essentially equal probability of linkage to the trait (with a LOD score=3.1, the maximum possible given the family structure). We have performed mutation analysis of six candidate genes that are expressed in the central nervous system or have known or potential roles in neuronal function, without detecting pathogenic mutations.

Discussion and conclusions: Clinically, this syndrome suggests the existence of a fourth type of JRALS, with features of spasticity, limb denervation and amyotrophy, and bulbar involvement. Our results can be interpreted to support the identification of one true and one false locus, and mathematical modeling of our linkage strategy suggests a 15% probability of this possibility. Alternatively, they can be interpreted to suggest digenic inheritance. Screening of additional candidate genes at each locus is underway. If the syndrome is monogenic, identification of additional families should help to distinguish which of the two autosomal loci contains the disease gene. Characterization of the genetic defect may shed light on mechanisms of motor neuron death, particularly as those mechanisms occur in the more common forms of motor neuron disease.

P225 ANTICIPATION IN A PEDIGREE WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS SECONDARY TO A MUTATION IN SUPEROXIDE DISMUTASE TYPE 1 (V148G)

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Background: Current descriptions of kindreds with familial amyotrophic lateral sclerosis (fALS) secondary to mutations in superoxide dismutase type 1 (SOD1) argue that genotype determines phenotype. fALS kindreds secondary to mutations in SOD1 published thus far in the literature suggest that the age of onset, site of onset and survival is similar for a given SOD1 mutation. One previous Italian study demonstrates generational anticipation, where the age of onset is younger with each successive generation in the setting of L84F SOD1 gene mutation (1).

Objective: The aim of this study was to document generational anticipation and variable phenotype in a single kindred with V148G mutant SOD1.

Methods: Family history was obtained for a large kindred in New South Wales with fALS secondary to a V148G mutation in SOD1. Site of onset, age at onset and survival was determined for carriers over three generations.

Results: In an ongoing investigation, ten patients with fALS have been identified in a large kindred in New South Wales. All affected patients in the kindred who have been tested have demonstrated the V148G mutation in SOD1. In the first generation, the age of onset ranged between 60 and 75 years, with bulbar and limb onset. The second generation developed symptoms between 45 and 60 years, with both limb and bulbar onset. The third generation developed symptoms between the ages of 19 and 30 years, with primarily bulbar onset of disease. The duration of survival varied widely between the three generations.

Conclusion: This kindred demonstrates the phenomenon of generational anticipation, described only once before in fALS secondary to mutations in SOD1. Furthermore, the concept of genotype determining phenotype is not

concordant with our kindred. This kindred suggests that other disease modifiers exist that determine age at onset and site of onset in fALS secondary to mutations in SOD1.

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P226 FURTHER SCREENING OF SUPEROXIDE DISMUTASE 1 GENE MUTATIONS IN CHINESE SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder involving motor neurons in motor cortex, brain stem and spinal cord. Ninety percent of patients are sporadic (SALS) and 10% are familial (FALS), with multiple autosomal dominant and recessive forms. Currently three major ALS genes and five additional loci have been identified. The first gene (ALS1) associated with adult-onset autosomal dominant form of the disease encodes for the cytoplasmic Cu/Zn superoxide dismutase (SOD1). Approximately 20% of FALS cases and 2% of overall ALS cases have identifiable mutations in this gene. At the present time more than 110 different mutations located in all five exons of the gene have been identified worldwide. We have reported our primary screening result of SOD1 gene mutations in sporadic cases with three new mutations found in 63 cases, but no definite genotype-phenotype relation was identified.

Objectives: We performed a further screening of the distribution of SOD1 gene mutations in a new larger cohort of Chinese ALS patients in order to assess the frequency of SOD1 gene mutations in SALS cases. Clinical features of patients with mutated SOD1 gene are reported.

Methods: Blood was collected from 48 patients with sporadic ALS and one familial patient. The El Escorial diagnostic criteria were used. Genomic DNA was prepared from blood using standard procedures. PCR amplification of five exons and introns was performed using primers as previously described. The reaction product was sequenced on double strand with an automated DNA sequencer (ABI 377) using the Big dye terminator cycle sequencing premix kit.

Results: H46R mutation was found in one familial patient with one hundred members. No mutations were found in 48 sporadic patients.

Discussion and conclusions: This study reports screening of gene mutations of SOD1 in Chinese sporadic and familial ALS patients. The H46R mutation is commonly found in Asia not in North America and Europe. No more mutation was identified in sporadic patients. The frequency of SOD1 gene mutations in SALS cases was comparable with that found in other surveys in different cohorts all over the world.

P227 POLYMORPHISM OF A SOD1-MUTATION IN A LARGE ALS FAMILY

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Background: Mutations in the Cu/Zn superoxide dismutase gene (SOD1) are well-known causes of amyotrophic lateral sclerosis (ALS) and account for approximately 20% of familial ALS cases. While reduced penetrance of SOD-associated ALS is not unusual, there has been no description of a gene polymorphism of a SOD1 mutation in one family.

Methods: We describe a large family with 13 members that suffered from ALS. All affected members of the family that were alive, could be contacted and gave informed consent for genetic testing were examined. DNA of those individuals and some not affected family members was extracted and tested for mutations in the SOD1 gene.

Results: The clinical presentation of all affected individuals was comparable with slow progression and predominant signs of the second motoneuron. Out of 5 tested affected individuals 2 had an E100K mutation of the SOD1 gene, whereas the 3 remaining did not have a SOD1 mutation. Out of 5 healthy family members, one individual carried the mutation.

Discussion: We could not find any correlation between the E100K mutation and clinical parameters. We conclude that the SOD1 mutation is not the main pathogenetic factor in this family to cause ALS. Our results contribute to the ongoing discussion concerning the relevance of SOD1 mutations in the pathogenesis of ALS and support the hypothesis of a heterogeneous cause of the disease, where the SOD1 mutation is only one of many pathogenetic factors.

P228 USING DHPLC TO DETECT SOMATIC MUTATIONS IN ALS

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Background: A genetic alteration in an early embryonic progenitor cell can cause a somatic mutation. This

mutation might be preferentially represented in tissues that arose from that cell line. Recently, a somatic mutation was identified in a case of early-onset Alzheimer disease that was present in only 14% of cells in the cerebral cortex. Mutations such as these are not detected using traditional methods of DNA sequencing. Denaturing high performance liquid chromatography (DHPLC) is a sensitive technique for detecting gene mutations. The sensitivity of DHPLC makes it ideal to detect low levels of somatic mutation in CNS tissue.

Objective: To develop a DHPLC assay to detect somatic mutations that could be present in low levels in brain tissue of patients with sporadic ALS.

Methods: A heterozygous mutation in exon 4 of *SOD1* was mixed with varying amounts of a wildtype genomic DNA, resulting in concentrations of mutated DNA from 2.5% – 50%. After PCR, the mutated and wildtype DHPLC profiles were superimposed. Fractions of all mutant peaks detected were collected to isolate the mutant signal. These fractions were re-amplified to enrich the mutation signal. Samples were sequenced before and after fractionation and the presence of mutant signals was compared.

Results: Sequencing before DHPLC fraction collection could readily detect the *SOD1* mutation in 50% and 25% of the DNA template. After mutation-enrichment with fraction collection, the 10% mutant signal was now also detectable on sequencing. Although DHPLC could detect a mutant peak when present in as little as 5% of the total DNA, one round of fraction collection and re-amplification alone was insufficient to confirm the mutation at this concentration.

Discussion and conclusions: These results show that DHPLC is a sensitive tool for detecting low levels of mutation in the CNS. Re-amplification of the fractions collected from the mutant peaks enhance the mutant signal and increase the sensitivity for sequencing. A second round of fraction collection would further increase sensitivity. This technique would be of use to screen candidate genes in the CNS of ALS patients for low levels of somatic mutation.

P229 SCREENING OF GENES THAT HAVE AN IMPORTANT ROLE IN THE DEVELOPMENT OF MOTOR NEURONS IN ALS PATIENTS

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Background: SOD1 is a highly-expressed gene in the brain that, when mutated, yields a remarkably selective phenotype only in motor neurons. This may be due to genetic or physical interactions with genes specific to this population of neurons. A set of genes specifically expressed at different stages of development in mouse corticospinal motor neurons (CSMN) has been recently identified, including 29 statistically significant and biologically relevant genes (1). Several of these key genes are potentially instructive for CSMN development while others are exclusively expressed within the CSMN. We hypothesized that they represent candidate genes which may be mutated in ALS patients. Interactions of these genes with SOD1 could help explain the specificity of neuron-targeting in ALS.

Objectives: To screen ALS patients in order to identify novel mutations in a subset of genes specifically expressed in CSMN. This may help to implicate genes crucial to motor neuron development as causative in ALS.

Methods: Following a complete annotation of 29 CSMN-specific genes, the entire exonic sequence of these genes was sequenced directly in 190 *SOD1*-negative familial ALS patients. Polymorphisms of these genes were eliminated by comparing them with SNP databases and by comparative sequencing of 190 ethnically matched controls.

Results: Exons and intronic boundaries of 12 genes were entirely sequenced in 190 ALS patients. A total of 54 variants were identified, including 16 intronic, 25 synonymous, and 13 nonsynonymous changes. Notably, no frame-shift, nonsense or splice-site mutations were identified. Of the missense changes, 5 are known SNPs, 2 are common changes, and 6 are rare variants identified in 5 separate genes. Of the rare variants, 2 are predicted to potentially harm the function of the protein. Follow-up examination of these genes in additional ALS and control samples will help to determine which one(s) are significant to ALS.

Conclusions: A complete sequence of all 29 CSMN genes in ALS patients will reveal several mutations causing deficits in the development of motor neurons. In the future, we plan to functionally characterize these mutations in *in vitro* and *in vivo* models.

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P230 PARAOXONASE GENE POLYMORPHISMS ARE ASSOCIATED WITH ALS IN THE FRENCH CANADIAN AND SWEDISH POPULATIONS

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Background: A variety of genes ranging in function from cytoskeletal structure to oxidative stress response have been represented in association studies with respect to amyotrophic lateral sclerosis (ALS). Notably, two such studies reported an association to ALS through variants in the paraoxonase (PON) gene cluster (1,2). The PON cluster consists of three adjacent genes on chromosome 7q21.3 that aid in the detoxification of organophosphate insecticide and in preventing the oxidation of lipoproteins.

Objectives: We sought to test the frequency of coding and intronic PON polymorphisms in the founder French Canadian population of Quebec, as well as a larger subset of sporadic ALS patients in France.

Methods: We collected 532 ALS cases and 465 controls from France, 550 cases and 512 controls from Sweden as well as 94 SALS cases and 94 controls from Quebec. A further 94 Parkinson Disease cases from Quebec were sampled for comparison of the results with a separate neurological disease population. Five single nucleotide polymorphisms (SNPs) were selected for analysis, including three coding SNPs and two intronic SNPs reported previously to be associated with ALS (2). Taqman genotype asays were performed. Haplotype and statistical analysis was performed using Unphased v5.1.26.

Results: No individual SNPs were found to be correlated in either population; however, a haplotype analysis of variants surrounding the PON1 gene yielded a disease haplotype specific to ALS patients from Quebec ($p < 1 \times 10^{-4}$). Individuals with Parkinson Disease did not demonstrate a similar haplotype. Similarly, a haplotype of SNPs in PON2 showed significant association in the Swedish population.

Conclusions: We have identified two populations where susceptibility to ALS is significantly associated with variants in the PON gene cluster, which extends the PON gene association study results reported by other groups. The functional consequence of the variants identified with respect to motor neuron degeneration will provide insight into the role of these genes in susceptibility to ALS.

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P231 PARAOXONASE 1 (PON1) ORGANOPHOSPHATE HYDROLYSIS IS MINIMALLY INCREASED IN ALS

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Background: Five recent studies report a genetic association of the paraoxonase locus with sporadic amyotrophic lateral sclerosis (ALS). The functional significance of this association has not yet been determined.

Objective: We tested the association of paraoxonase 1 (PON1, MIM 168820) DNA variants with PON1 hydrolysis of organophosphates and compared PON1 enzyme activity in ALS subjects and controls.

Methods: 158 sera from ALS participants, 158 age, race and gender matched controls, and 30 matched cerebrospinal fluid samples were tested for paraoxonase, diazoxonase and arylesterase activity. Participants with ALS were genotyped using tagging single nucleotide polymorphisms (tag-SNPs) across the PON locus and survival data were correlated with enzyme activity and genotype.

Results: There was a trend towards increased paraoxonase activity in ALS compared to controls (mean control paraoxonase 710.12 $\pm$ 471.71 U/L, mean ALS 823.9 $\pm$ 586.46 U/L, $p=0.056$ after correction) which strongly correlated with increased frequency of the $PON1_{R192}$ variant ($p=0.028$). There was no significant difference in PON1 protein levels, arylesterase or diazoxonase activities. Similarly, we found that organophosphate hydrolysis rates had no effect on survival or site of onset. The minor allele of intronic PON1 rs2074351 was associated with reduced survival ($p=0.045$), but did not affect any of the enzyme activities tested. SNP variants that determined organophosphate hydrolysis in our samples had no effect on survival or site of onset.

Interpretation: Contrary to expectations, PON1 paraoxonase, diazoxonase and arylesterase activities are not reduced in ALS. The increase in $PON1_{R192}$ frequency in our study supports previous genetic susceptibility studies. Our findings suggest that the influence of PON1

polymorphisms on ALS susceptibility is not due to reduced organophophate hydrolysis. This is the first study to test the function of PON1 in ALS.

P232 SEX HORMONE RECEPTOR POLYMORPHISMS ARE NOT ASSOCIATED WITH ALS

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Background: In ALS, there is a predominance of males compared to females and women appear to have a shorter reproductive period due to a later menarche and earlier menopause. This suggests that sex hormones are involved in ALS pathophysiology. Sex hormones have beneficial effects on motorneurons *in vivo* and *in vitro* and have been put forward as a potential therapy in ALS. Since the actions of sex hormones are mainly exerted via their receptors, we sought to determine whether the sex hormone receptor genes are susceptibility factors for ALS.

Objective: In this study, we screened the main sex hormone receptor genes in humans, the androgen receptor (AR) and estrogen receptor-α (ERα) and ERβ for genetic variations.

Methods: We re-sequenced the coding regions in a limited number of patients and controls and the detected variations were further investigated in a larger cohort of Dutch ALS patients and sex-, age,- and gender matched controls using Taqman assays.

Results: A total of 6 SNPs were detected in the ERα and ERβ. None of these were associated with ALS susceptibility. Also, there was no significant relation with age at onset, site of onset or survival. For the AR, we could not detect an association between a frequent SNP and ALS. The numbers of CAG repeats in the first exon of the AR gene were not significantly different compared to controls.

Conclusion: In conclusion, we found that the sex hormone receptors, ERα, ERβ and AR, are not susceptibility factors for ALS.

P233 ASSOCIATION BETWEEN RNASE A SUPERFAMILY MEMBERS AND SPORADIC ALS

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Background: We have previously reported seven missense mutations of the RNase A superfamily member *ANG (RNASE 5)* in familial ALS, and a common haplotype across the *ANG* locus in sporadic ALS (SALS). These mutations are predicted to affect protein folding, resulting in lower ribonuclease activity of angiogenin. Angiogenin is one of a large family of enzymes which exhibit ribonuclease activity, and which have been grouped as the RNase A superfamily. To date 13 genes of the RNase superfamily have been described, forming a cluster on chromosome 14q11.2.
Purpose: To screen other members of the RNase superfamily for association with sporadic ALS, using a screening and a confirmation phase.

Methods: Using HapMap Project phase II and Haploview tagger (v 3.32) we selected 39 tgSNPs to cover the common genetic variation across each RNase gene and 2 kb of flanking sequence. The study population comprised 282 Irish patients with typical SALS and 280 unrelated control subjects. Genotyping of all 39 SNPs was performed using modified Taqman chemistries in 180 case-control pairs. SNPs found associated in the screening phase were genotyped in a further 100 case-control pairs. Statistical analyses and haplotype estimations were conducted using Haploview and UNPHASED software. Haplotype associations are correction for multiple testing by permutation.

Results: A common haplotype, AATAGTG, across *RNASE 11* was overrepresented among cases vs controls (13.5% vs 8.1%, $p=0.06$ in the screening population; 14.3% v 9.2%, $p=0.2$ in the confirmation population; 13.9% v 8.5%, permuted $p=0.01$ overall). This haplotype resides on a separate block to *ANG*.

Conclusions: These data support the hypothesis that common variation across RNase superfamily members may increase susceptibility for SALS.

P234 GENETIC ASSOCIATION OF POLYMORPHISMS IN THE CHGA AND CHGB GENES WITH ALS

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Amyotrophic lateral sclerosis is a degenerative neurological disorder. At present, only the SOD1 gene has been clearly shown to predispose to classical dominant late onset ALS. While this discovery has lead to significant insights into the causes of ALS, the basic pathogenic mechanisms remain unknown. Recently, a specific interaction between chromogranin proteins (CgA and CgB) with different SOD1 mutants has been described. This led us to screen for sequence variants of the *CHGA* and *CHGB* genes in a cohort of ALS French patients from France and Quebec. We identified polymorphic variants of both genes occurring at higher frequency ($P < 0.001$) in ALS cases as compared to unaffected controls. Furthermore, we identified a novel missense variant changing a histidine to an arginine in the *CHGB* gene. This new missense variant was not found in controls and is predicted to alter protein structure. Biological validation and replication of our findings in the Swedish population are currently underway.

P235 CANDIDATE GENE ANALYSIS AMONG A LARGE FAMILIAL ALS COHORT

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Background: Around 10% of ALS cases are familial. Inheritance of familial ALS is usually autosomal dominant although rare recessive and sex-linked forms also exist. Genetic analyses of autosomal dominant ALS families have identified several genes, including *SOD1*, and *DCTN1*. Several other genes have been implicated as pathogenic or susceptibility genes. However, these genes combined only account for about 20% of familial ALS cases (about 2% of all cases of ALS). Therefore the majority of ALS genes remain to be identified. To date, only a small number of sporadic ALS cases have been found with putative risk associated DNA polymorphisms or gene mutations. However it is far from clear whether these sequence variations are truly risk or disease causing as they are difficult to distinguish from normal polymorphisms. The precise role of these variations and whether they are truly risk or disease causing mutations needs to be established by functional assays or by showing that they also cause familial ALS.

Objectives: Our aim is to screen a large cohort of ALS families for mutations in putative disease genes, and to perform functional assays to establish whether there is support for their proposed pathogenic role.

Methods: We investigated a cohort of 109 Australian ALS families. Mutation analysis of candidate genes in ALS families was performed using direct DNA sequencing of all exons and flanking sequences amplified from patient genomic DNA.
For expression constructs, full-length cDNA was amplified by RT-PCR and cloned into expression vector pcDNA-3.1. Putative mutations were introduced into the cDNA sequence using site-directed mutagenesis. Constructs were transfected into neuroblastoma and motor neuron like cell lines. Protein expression was analysed by Western blot and immunostaining.

Results: Genes previously implicated in the pathogenesis of ALS were screened in our cohort of ALS families. These included superoxide dismutase 1 (*SOD1*) Dynactin (*DCTN1*), Angiogenin (*ANG*), and chromatin-modifying protein gene 2B (*CHMP2B*). *SOD1* mutations were identified in 17 families (15.6%). Three putative *ANG* mutations were identified in three ALS families (2.7%). No mutations in *DCTN1* or *CHMP2B* were identified.
Of the putative *ANG* mutations, one is novel. An expression construct incorporating this novel *ANG* mutation has been prepared and is currently being analysed in neuronal cell lines.

Discussion: Around 16% of families were *SOD1* positive which is in accordance with previous estimates. Putative *ANG* mutations were also identified, however DNA was unavailable from additional affected individuals such that segregation with disease could not be confirmed. Functional studies are required to establish whether these *ANG* mutations are truly pathogenic. No *DCTN1* or *CHMP2B* mutations were identified indicating that these are rare causes of familial ALS.

P236 ANGIOTENSIN-I CONVERTING ENZYME INSERTION/DELETION POLYMORPHISM IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Amyotrophic lateral sclerosis is a fatal motor neuron diesase characterized by the death of motor neurons in the brain, brainstem and spinal cord, leading to fatal paralysis. ALS usually begins with asymmetric involvement of the muscles in middle adult life. Almost 10% of ALS cases are familial.
Angiotensin I-converting enzyme plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II, a potent vasopressor, and aldosterone-stimulating peptide, and is also able to inactivate bradykinin, a potent vasodilator. It appears that there is evidence indicating that ACE I/D polymorphism may play a role in the etiopathogenesis of some neurodegenerative diseases. Therefore we wanted to know whether ACE I/D polymorphism is associated with sporadic ALS in Turkey

Objectives: To examine whether angiotensin I-converting enzyme gene I/D polymorphism is associated with patients with sporadic ALS.

Methods: We studied 187 patients with sporadic ALS (SALS) (78 women 41.7%; 109 men 58.3%; age range 26–89 years , 62.82±15.34 years), and 287 healthy controls(119 women 41.5%; 168 men 58.5%; age range 26–90 years; 60.09 ±17.16 years). All patents diagnosed with SALS were examined by a neurologist and met the revised El Escorial criteria for the diagnosis of clinically definite or probable ALS. A polymerase chain reaction was carried out on the genomic DNA samples isolated from blood. The PCR product was a 190 bp fragment when it was D allele. When the fragment was a 490 bp, it was I allele. The electrophoresis was performed on a 10% polyacrylamide gel followed by silver staining.

Results: The ACE D allele frequency was 60.16% in cases and 62.02% in controls. The ACE I allele frequency was 39.84% in cases and 37.98% in controls. In addition, the distributions of the ACE DD , ID and II genotypes were 39.6%,41.2%,and 19.3% in cases and 41.8%, 40.4%, and 17.8% in controls respectively. There was no association between ACE I/D polymorphism and SALS (χ^2=0.290df2P=0.865).

Discussion and conclusions: It has been reported that ACE I/D gene polymorphism is associated with Alzheimer's disease. However, in our cohort study, this is the first study to show that there is neither allele nor genotype association between ACE I/D gene polymorphism and SALS in Turkey

P237 ASSOCIATION OF POLYMORPHISMS IN VASCULAR ENDOTHELIAL GROWTH FACTOR GENE WITH THE AGE OF ONSET OF AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: This study investigated the association between polymorphisms in vascular endothelial growth factor (VEGF) gene (-1558C-T, -1190A-G and -1154A-G) and age at onset of amyotrophic lateral sclerosis (ALS).

Methods: Between July 2000 and June 2004, we conducted a clinic genetic study at Peking University Third Hospital, China. The analyses included a total of 100 ALS subjects. Genotyping was performed by using the 5′-nuclease assay technology (Applied Biosystems) with TaqMan® allele specific fluorogenic oligonucleotide probes. We used multiple linear regression modeling and Haplotype-based association test to analyze the association of *VEGF* gene polymorphisms with the age of onset, adjusting for initial symptoms and sex.

Results: The results indicated the patients with the -1190A/G and -1190G/G genotypes exhibited about a 4.1 and 9.4 years greater shortening of age of ALS onset than did the patients with the -1190A/A genotype. A similar pattern emerged when *VEGF* -1154A-G gene was considered: the β was −7.9(SE, 1.9) years and −11.7(SE, 3.0) years for -1154A/G and -1154G/G genotypes, respectively. Whereas, the *VEGF* -1558C-T had a positive effect in the -1558C/T group (P=0.007, β=7.0) and -1558T/T (P<0.001, β=9.6) compared to -1558C/C group. We did neither observe an interaction, nor haplotype association with age of onset among -1558C-T, -1190A-G and -1154A-G.

Conclusions: Our results indicate, for the first time, that there was an important association between the polymorphism of *VEGF* gene and age of ALS onset. This suggests a possible role for *VEGF* variability in the etiology of individual differences in age of onset.

P238 LACK OF ASSOCIATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ANGIOGENIN GENE POLYMORPHISMS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN THE POLISH POPULATION

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Background: Genetic factors involved in the pathogenesis of sporadic amyotrophic lateral sclerosis (sALS) still remain largely unknown. A candidate gene might be vascular endothelial growth factor (VEGF), which is a major factor for normal and pathological angiogenesis, but it also directly affects the neuronal cells and regulates neuronal blood flow. The recent studies showed that -634 G/C and -2578 C/A polymorphisms of VEGF gene were associated with a greater risk of sALS. Angiogenin (ANG), an angiogenic member of the RNase A superfamily, bears striking similarity to VEGF. The analysis of the ANG gene demonstrated a significant allelic association with the rs11701 single nucleotide polymorphism (SNP) and identified a novel mutation in sALS cases.

Objective: We have studied a possible association between -634 G/C and -2578 C/A polymorphisms of VEGF gene and -2138T/G polymorphism of ANG gene and the risk factor of sALS in the Polish population.

Material and methods: We have included 204 unrelated patients with sALS and 348 unrelated healthy controls matched for age and sex for VEGF gene polymorphisms evaluation and 203 unrelated patients with sALS and 220 unrelated healthy controls matched for age and sex for ANG gene polymorphism evaluation. The definite or probable diagnosis of ALS was established according to El Escorial Criteria (1998) in Krakow MND Center. The polymorphisms were studied by polymerase chain reaction (PCR) and restricted enzyme digestion.

Results: VEGF -634 and VEGF -2578 genotype frequency in patients with sALS (GG-0.57, GC-0.36, CC-0.07; CC-0.22, CA-0.51, AA-0.27) and controls (GG-0.53, GC-0.38, CC-0.09; CC-0.23,CA-0.52, AA-0.25) were very similar and did not influence the risk of occurrence of sALS ($p=0.57$; $p=0.81$, respectively). ANG-2138 genotype frequency in patients with sALS (TT-0.78, TG-0.20, GG-0.02) and controls (TT-0.73, TG-0.27, GG-0.05) were also very similar and did not influence the risk of occurrence of sALS ($p=0.09$).

Conclusions: Although some previous studies discovered an association with polymorphisms in the VEGF gene and risk of sALS in a few European populations we demonstrated that -634 G/C and -2578 C/A polymorphisms of VEGF gene are not connected with the greater risk of sALS in the Polish population, which was also shown in German, Dutch and London populations. We found also that -2138T/G polymorphism of ANG gene is not connected with the greater risk of sALS in the Polish population.

P239 SCREENING HYPOXIA GENES IN SPORADIC ALS IN TWO EUROPEAN POPULATIONS

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Background: Genetic variations in the two hypoxia-inducible angiogenic genes, *VEGF* and *ANG,* have been linked with sporadic amyotrophic lateral sclerosis (SALS). It is likely that these variations result in either reduced levels or functioning of these factors. *VEGF* and *ANG* belong to a larger group of angiogenic genes which are upregulated under hypoxic conditions, and which have been suggested to exhibit co-dependency, with each providing a necessary component to the overall biological response. This implicates a potential role for other similar hypoxia-responsive genes acting upon a common pathway that is crucial to motor neuron survival.

Purpose: To screen other hypoxia-inducible genes for association with SALS in two European populations.

Methods: We selected 114 tagging single nucleotide polymorphisms (tgSNPs) from the HapMap CEPH panel to capture the common genetic variation across a group of 16 *VEGF*-like and 8 *ANG*-like hypoxia-inducible genes. In the first phase, genotyping of these tgSNP was performed in 227 individuals with typical SALS and a group of ethnically matched controls from the Irish MND DNA Bank. To discriminate among false positive findings, tgSNPs showing evidence of association in the Irish are being screened in 311 Swedish SALS cases and 293 controls.

Results: In the Irish SALS population, multiple marker associations were identified in *inhibin beta A (INHBA)* (rs2237432, rs3801158; $p=0.02$, $p=0.046$). Single marker associations were identified in *ANGPTL4* (rs1044250; $p=0.048$), *CAV1* (rs3807986, $p=0.041$), *STC2* (rs3756530, $p=0.037$) and *osteopontin* (rs6532040, $p=0.04$). We are currently screening for replication in the Swedish SALS population.

Conclusions: Hypoxia-responsive genes are attractive candidates as susceptibility factors for SALS. Replication in the Swedish dataset will determine the importance of observations in the Irish.

P240 ANGIOGENIN: A NOVEL CANDIDATE GENE FOR ALS PATHOGENESIS IN THE ITALIAN POPULATION

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Background: Most ALS cases (90%) are sporadic. Mutations in SOD1 gene account for only about 20% of familial ALS cases and 3–6% of sporadic ones. Mutations in alsin, VAPB, SETX, and dynactin genes are rare. Angiogenin (ANG), as VEGF, has been recently described as a novel candidate gene for ALS. ANG, an angiogenic growth factor up-regulated by hypoxia, is highly expressed in motor neurons. Recent experimental data demonstrate angiogenin involvement in neurite path-finding and out-growth during neural development. Missense mutations in ANG gene have been identified in Northern European (Irish/Scottish and German) patients both in sporadic (sALS) and familial (fALS) cases negative for SOD1 gene mutations. A significant allelic association with the rs11701 single nucleotide polymorphism (SNP) has also been described in the Irish and Scottish ALS patients.

Methods: We screened 738 Italian ALS patients and 517 controls for ANG coding, 3′UTR and 5′UTR sequences using both dHPLC and direct sequencing procedures.

Results: We found no association with the previously reported rs11701 SNP in our ALS patients. Interestingly, in contrast to the negative results of two recent Italian mutational screening studies, we identified seven different mutations in 14 patients (8 sALS and 6 fALS). Six out of seven were novel missense mutations, three of which were located in the signal peptide region, two in the coding sequence and one in the 3′UTR. In addition we detected the previously described I46V mutation in six patients and in one control.

Discussion: Our results confirm the presence of mutations in ANG gene also in the Italian ALS population. Although functional evidences of ANG mutations are still missing, angiogenic growth factors seem to have an important role in the pathogenesis of ALS.

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THEME 10 EPIDEMIOLOGY

P241 ANGIOGENIN LEVELS IN CSF AND SERUM

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Background: Recent studies of VEGF in ALS have established that hypoxia responsive proteins may be important both in disease pathogenesis and as possible therapeutic options. Mutations in *ANG*, which codes for a related hypoxia responsive protein, angiogenin, have been identified in familial ALS, and multiple ANG haplotypes are associated with sporadic ALS in the Irish population. We have also recently reported that serum angiogenin levels in Irish ALS patients differ from controls.

Objectives: 1) To determine whether angiogenin is present in cerebrospinal fluid (CSF), and to quantify the range of angiogenin levels in CSF in ALS patients and controls. 2) To investigate the relationship between levels of CSF and plasma angiogenin levels.

Methods: CSF from 196 Swedish patients with ALS was analysed. All patients fulfilled the El Escorial criteria for probable or definite ALS. Samples were compared with control CSF from 171 unrelated, ethnically matched controls with no known history of a neurologic disorder. Commercially available kits (Quantikine, R&D Systems, Abingdon, UK) were used to perform in vitro enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of angiogenin.

Results: Angiogenin is present in CSF. The medians were not significantly different between control and patient groups ($p=0.677$). When stratified for gender, a statistical difference was observed, with levels that were 13% higher in males than females ($p<0.003$). A trend towards higher levels of angiogenin was seen with increasing age ($p<0.01$). Plasma angiogenin levels were significantly different between the control and patient groups ($p<0.0005$). The median values of angiogenin were higher in the control group compared to the patient group. Levels increased with age ($p<0.018$). A weak correlation was noted between CSF and plasma angiogenin concentrations ($r=0.277$, $p<0.0005$).

Conclusion: Angiogenin is expressed in CSF. Levels correlate with age and gender. No overall differences in CSF angiogenin levels were observed between ALS and control groups. Contrary to our previous findings, angiogenin levels in plasma were lower than controls in the Swedish cohort. These findings support the hypothesis that angiogenin, like VEFG, is a biologically important protein in ALS.

P242 MONOCLONAL GAMMOPATHY IN ALS AND OTHER MOTOR NEURON DISORDERS

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Background: Several lines of experimental data suggest that immunological factors may play a role in the pathogenesis of motor neuron degeneration. Previous studies reported higher occurrence of monoclonal gammopathy in patients with motor neuron disease (MND), in particular when clinical symptoms of peripheral motor neuron damage were present. However, these findings should be interpreted with caution due to methodological limitations, such as selection bias and lack of application of a standardized sensitive assay for detection of monoclonal immunoglobulins in serum.

Objectives: To compare the frequency of monoclonal gammopathy in patients with ALS to controls; to compare the frequency of monoclonal gammopathy in patients with other motor neuron diseases to controls.

Methods: Between 1 January 1999 and 1 February 1 2007 patients diagnosed with ALS (n=445), progressive spinal muscular atrophy (PSMA) (n=117), spinal muscular atrophy (SMA) (n=59), primary lateral sclerosis (PLS) (n=95) were included. 450 age- and sex-matched volunteers were recruited for the control group. Screening for the presence of monoclonal protein in serum was performed by agarose electrophoresis and consequent immunofixation of suspected bands.

Results: 14.5% of the patients with PSMA had monoclonal protein in serum compared to 7.4% of the controls (OR 2.1; 95% CI=1.1–4.0). Compared to the control

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group, patients with ALS, PLS and SMA had similar frequency of monoclonal gammopathy.

Conclusions: Monoclonal gammopathy occurs more frequently in patients with PSMA. Immunological factors may play a role in PSMA: the peripheral motor neuron may contain a target or an antigen for a monoclonal antibody response.

P243 LEVEL OF HYPERMETABOLISM IS SIGNIFICANTLY INCREASED IN FALS AS COMPARED WITH SPORADIC CASES

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Background: An abnormally elevated level of resting energy expenditure (REE, measured by indirect calorimetry) has been reported in a subset of patients with amyotrophic lateral sclerosis (ALS). Interestingly, hypermetabolism (measured REE/calculated REE$\geq$1.1, or 110%) has also been observed in transgenic mice harbouring ALS-causing mutations in the *SOD1* gene.

Objective: We tested whether patients with familial ALS (FALS) had a level of hypermetabolism differing from that of patients with sporadic ALS (SALS).

Methods: Eleven patients with FALS (from 10 different families, all negative for the screening of *SOD1* mutations by direct sequencing) who had performed an indirect calorimetry in our centre were retrospectively identified. As a control group, we used a sample of 33 patients with SALS, matched for age and sex with the FALS patients.

Results: 11/11 (100%) patients with FALS were hypermetabolic, compared to 17/33 (52%) patients with SALS ($p=0.009$). The mean level of hypermetabolism was significantly higher in FALS patients (124 ± 8 %, than in SALS patients (113 ± 12 %, $p=0.01$).

Discussion and conclusion: Subjects with FALS are supposed to carry mutations or strong genetic risk factors predisposing to the disease, which may also be responsible for the higher REE observed in these patients. In the absence of infection, inflammation or hyperthyroidism, hypermetabolism may result from mitochondrial uncoupling. Several other lines of evidence support the occurrence of a mitochondrial dysfunction in the course of ALS. Our results suggest that this mitochondrial dysfunction is genetically driven in patients with FALS and may therefore be directly involved in the disease pathogenesis.

P244 ALS, PHYSICAL ACTIVITY, TRAUMA, AND SPORT. A PILOT CASE-CONTROL STUDY

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Background: A high risk of ALS has been recently observed in professional soccer players in Italy and then confirmed in some case-control studies but not in others. Repetitive trauma, heavy physical exercise and drug abuse have all been indicated as risk factors.

Objective: To assess the correlation between amyotrophic lateral sclerosis (ALS) and physical activity, (repeated) traumatic events, and (organized) sports.

Methods: A population-based case-control study was activated in three European countries (Italy, Ireland, UK). Cases were patients with newly diagnosed definite, probable or possible ALS (El Escorial criteria). For each case, two age- and sex-matched controls were selected, mostly from the referent general practitioner's list. Using a semistructured questionnaire, cases and controls were asked about history of occupation(s), physical exercise, and sport(s). For each activity, duration and intensity of exposure was indicated. Traumatic events were recorded with type, number and site of injury, and complications. Drug exposure (type and duration) was also recorded. An interim analysis (presented here) was pre-planned after enrolment of about 50 cases and 100 controls, to test the feasibility of the study design.

Results: The sample included 59 cases and 112 controls (Italy, 103; Ireland, 23; UK, 45). Blue collar workers were 40.7% (cases) vs 17.9% (controls)(OR 3.1; 95% CI 1.5–6.8). The mean number of years spent with any occupation was 28.4 in ALS patients and 22.1 in controls ($p<0.05$). The mean number of years spent doing any sport was 9.8 in cases and 5.3 in controls ($p<0.05$). ALS patients practiced strenuous physical exercise during work more than controls (11.9 vs 3.6%; OR 3.6; 95% CI 0.9–15.6). Strenuous sport-related physical exercise was no different in cases and controls (11.9 vs 17.9%). Three patients (no controls) reported a history of professional sport (soccer 2; athletics 1; $p<0.05$). Traumatic events (single or repeated) and drug exposure were no different in cases and controls.

Conclusions: Physical activity and professional sports may be risk factors for ALS independent of trauma and drug abuse.

P245 ALS AND SOCCER: THE EPIDEMIC IS STILL ONGOING AND IS SOCCER-SPECIFIC

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Background: A six-fold increase of incidence of ALS has been described in Italian professional soccer players between 1970 and 2001.

Aims: To update to 2006 the findings of the Italian professional soccer players cohort and to describe the observations in two other cohorts of Italian professional athletes (basketball players and road cyclists).

Methods: ALS cases were searched for in three cohorts of Italian professional athletes. The Italian professional soccer players cohort included all male professional soccer players who played between 1970 and 2001 (7,325 athletes); the Italian basketball players cohort included all male professional who played between 1980 and 2003 (1,973 athletes); the Italian road cyclists cohort included all male professionals who were engaged by a professional road team between 1945 and 2001 (1701 athletes). ALS cases were identified through multiple sources (death certificates, the archives of Italian ALS centres, the archives of the Italian ALS Association; information provided by media and web sites; self-reports by ALS patients or their relatives).

Results: In the Italian professional soccer players cohort 3 new cases of ALS were found in the period 2002–2006. Considering the 5 cases who developed ALS before 2002, a total of 8 ALS cases were identified. Since the expected number of cases was 1.24, the standardized morbidity ratio was 6.5 (95% c.i., 2.8 to 12.7; $p < 0.00001$). The mean age of onset of the cases was 42.4 years (SD 7.5; range 33–56). Six cases were midfielders (0.58 expected; SMR 10.34; 95% c.i., from 3.79 to 22.54; $p < 0.00001$). Subdividing the soccer players according to the period when they stated to play as professionals (<1980 vs. 1980–2001), a significant increase was found in both groups (<1980: observed 4, expected 1.05; SMR 3.8; 95% c.i. 1.1 to 9.6; 1980–2001: observed 4, expected 0.20; SMR 20.3; 95% c.i., 5.5–52.0). No ALS cases were identified in the basketball players cohort (expected, 0.14), and in the road cyclists cohort (expected, 1.82).

Conclusions: The increase of risk of developing ALS among Italian soccer players has been confirmed by the extension of the observation to 2006, since 3 new cases were found in the 2002–2006 period, whose age of onset ranged between 36 and 45. The increase of risk was particularly evident for players who played after 1980. The absence of cases in the other two cohorts of professional athletes indicates that the increase of risk for ALS among Italian soccer players is highly specific and not generically related to physical activity. A possible interaction between genetic and environmental factors can be hypothesized.

P246 HAS THE NATIONAL VETERANS AFFAIRS ALS REGISTRY CAPTURED ALL VETERANS WITH ALS? A UNIVERSITY AND VA ALS CLINIC-BASED VALIDATION STUDY

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Background: Beginning in 2003 a National Veteran Affairs (VA) ALS Registry attempted to define all cases of ALS in US military veterans. This registry included veterans of the active military and not the National Guard. At the University of Wisconsin Hospital and Clinics and the William S Middleton Memorial VA Medical Center, all ALS patients were ascertained as to whether they were military veterans, categorized as regular military with active duty or National Guard veterans, provided with a pamphlet explaining VA Cooperative Study 500A and asked to contact and register in the National VA ALS Registry.

Objective: To determine to what degree, the National VA ALS Registry entered US military veterans from these clinics and National Guard veterans and other motor neuron syndromes were excluded.

Methods: US military veteran ALS patients were monitored for their follow-up at the University ALS Clinic or VA ALS Clinic, entry into the National VA ALS Registry, completion of follow-up forms, requests for medical records, participation in VA DNA study, discharge from the study, death or loss to follow-up. Standard summary descriptive statistics were applied (Medicalc software, Mariakerke, Belgium).

Results: At the University ALS Clinic, 33 veterans (all males) with ALS (24–76 yr) were identified including 27 eligible active duty veterans (7 were transferred to the VA ALS Clinic), 1 National Guard active duty veteran and 5 National Guard veterans without active duty. Of 28 eligible active duty veterans, 20 (71.4%) entered into the National VA ALS Registry. Non-entry was due to not giving consent (2), too busy (3), not-re-contacted (3) and National Guard service only (5). At the VA ALS Clinic 19 additional veterans (18 males, 1 female) with ALS (34–77 yr) were identified. All VA ALS patients could be identified via CPRS but only 15 patients entered the National VA ALS Registry (78.9%). Including the 7 transferred ALS veterans 22/26 veteran ALS patients (84.6%) were entered into the Registry and 4 did not participate. The non-participants in the VA ALS Registry were twice as numerous at the University ALS Clinic (28.6%) compared with the VA Clinic (15.4%). In addition to those who did not participate an additional 5 National Guard veterans with ALS were not entered into the National VA ALS Registry. The National Guard ALS patients without active duty could have been an important internal control group experiencing military exposure but not being deployed outside their home state. Overall at

these clinics only 35/52 (67.3%) ALS patients with military experience were entered in the National VA ALS Registry. At the VA ALS Clinic, there were an additional 6 patients with bilateral or monomelic idiopathic amyotrophy. ALS, PMA MND, PLS syndromes comprised 52/58 (89.6%) of the motor neuron disease phenotypes seen in veterans at these clinics.

Discussion and conclusions: At these ALS Clinics with committed effort to enter all veterans with ALS and MND, up to 30.7% of patients with these clinical phenotypes both followed and not followed at the VA were not entered into the Registry.

P247 AMYOTROPHIC LATERAL SCLEROSIS AMONG 1991 GULF WAR VETERANS: EVIDENCE THE OUTBREAK IS ENDING

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Background: Recent reports established an elevated risk of amyotrophic lateral sclerosis (ALS) among 1991 Gulf War veterans. Two important questions remain to be answered: 1) Is the outbreak continuing or ending? 2) What is the etiology of the outbreak?

Objective: To address the first question, the distribution of disease onset times among the affected veterans was analyzed to determine whether the excess risk was time limited.

Methods: This analysis used data from a population-based case series identified from a nation-wide study of ALS among military personnel during the 10 years following the 1991 Gulf War and a subsequent 1 year surveillance period. Cases were identified from among the 2.5 million military personnel who were on active duty during the war using passive and active surveillance techniques. Among identified potential cases, disease status was verified by one or more of the following methods: medical record review, physical examination by a neurologist with expertise in motor neuron disease, or death certificate. Date of disease onset was defined as the case-reported date of onset of progressive weakness (which ultimately was shown to be due to ALS) that was recorded in the medical record. When missing, it was imputed using an algorithm derived from data where date of onset was known. Annual standardized incidence ratios (SIR) were calculated for all cases and for the subset with disease onset before age 45 years. Following standard occupational epidemiologic principles, the comparison group for the Gulf War veterans was the population of military personnel who were not deployed to S.W. Asia. The chi-square test of trend was used to test for monotonic trends in the annual SIR. The chi-square test of homogeneity was used to test for equality among the annual SIR.

Results: From 1991 through 2001, 124 cases occurred among the U.S. active duty military population. Forty-eight (48) cases occurred among the approximately 700,000 troops who had been deployed to the Persian Gulf region during the war; 33 (69%) of these cases had onset before age 45 years. The remaining cases occurred among the approximately 1.8 million troops who were not deployed to this region; 64% had onset before age 45 years. The annual SIR for deployed military personnel varied over time but did not demonstrate a monotonically increasing trend for either all cases ($\chi_1^2=0.11$; p-value=0.74) or for cases under 45 years of age at onset ($\chi_1^2=2.41$; p-value=0.12). A general increase in risk was observed with the highest risk occurring in 1996 and declining thereafter. Among military personnel who were not deployed to the Gulf region, no excess risk was observed at any time during the 11-year period.

Discussion and conclusion: The excess risk of ALS among 1991 Gulf War veterans appears to be limited to the decade following the war. Clues to the etiology of this outbreak may be gained by exploring exposures incurred by these veterans during their deployment.

P248 MILITARY VETERANS WITH ALS: FACTORS ASSOCIATED WITH SURVIVAL

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Background: The median survival of patients with ALS is 2–4 years from symptom onset, but there can be considerable variability from patient to patient. A few predictors of survival have been identified (e.g. older age at onset, non-extremity onset, shorter time to diagnosis), but much of this variability remains unexplained. Predictors of survival have not been specifically identified in veterans with ALS.

Objectives: The purpose of this study is to identify predictors of survival among a large American cohort of veterans with ALS.

Methods: We identified 1,085 subjects in the National Registry of Veterans with ALS who had definite or probable ALS. We examined various demographic, medical history, and military background variables to see if they were associated with ventilator-free survival time (determined from both the date of symptom onset and the date of diagnosis acquired by chart review). We censored any subject who had not died or had not started using a ventilator by 31 October 2006. We fit single variable Cox proportional hazard regression models to estimate hazard ratios for each of the predictor variables. Statistical significance was set a priori at α of 0.05.

Results: The cohort was primarily male (98%) and white (94%), with a median age of 59 years at symptom onset and 61 years at diagnosis. Median survival from date of symptom onset and date of diagnosis were 57.0 months (95%CI 52.0–62.0) and 40.0 months (95%CI 34.0–54.0), respectively. The following factors were significantly associated with reduced survival from date of symptom onset (determined using hazard ratios and 95% CIs): older age at onset (HR 1.39[1.29–1.50] per 10 year increase), non-extremity onset (HR 1.71[1.42–2.05]), cerebral vascular disease/stroke (HR 1.31[1.06–1.64]), and deployment to Vietnam (HR 1.35[1.11–1.63]). A longer time to diagnosis was associated with increased survival (HR 0.67[0.62–0.73] per year increase). All predictors were also significant using survival determined from diagnosis date.

Conclusions: In addition to verifying past predictors of increased survival (longer time to diagnosis) and reduced survival (older age at onset, non-extremity onset), we found that comorbid cerebral vascular disease/stroke and past deployment to Vietnam were also predictors of reduced survival. Additional research will be needed to understand the reasons for these new associations.

P249 NO EVIDENCE YET FOR INCREASED ALS RISK AFTER EXOGENOUS EXPOSURE TO CHEMICAL AGENTS AND METALS – A CRITICAL AND SYSTEMATIC REVIEW OF THE LITERATURE

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Background: Environmental exposures to chemical agents and metals are suggested to contribute to the risk of developing sporadic Amyotrophic Lateral Sclerosis (ALS). However, reports have been inconsistent. To summarize the data, two systematic reviews of the literature regarding chemical agents and metals according to the MOOSE guidelines were performed.

Methods: From MEDLINE, EMBASE, Cinahl, and Cochrane databases, methodology of selected studies was appraised according to Armon's classification system for ALS risk factor studies as well as quality of exposure. Studies meeting the validity criteria (methodological level of evidence (Armon I, II, III, or IV) and quality of exposure assessment (Exposure Assessment rating 3 or 4, indicating valid findings) were selected. Data for each chemical agent and metal were collected.

Results: 36 studies dealing with exposure to chemical agents in ALS patients were initially included; only 6 studies (1 case-control, 2 register-based case control, 3 register-based cohort) met the validity criteria. 49 studies dealing with exposure to metals were initially included; only 4 studies (1 case-control, 1 register-based case-control, 2 register based cohort) met the validity criteria. Risk estimates of most individual chemical agents and metals were reported in one to three studies. Risk estimates were reported in more than one study and were significantly increased risk for pesticides and selenium.

Conclusions: From numerous heterogeneous studies concerning chemical agents (n=36) and metals (n=49), a limited number of informative studies remained for chemical agents (n=6) and metals (n=4). Candidate chemical agents and metals could not be identified; perhaps more evidence for pesticides and selenium will be available when future studies have been performed.

P250 DETERMINATION OF VANADIUM IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: The cause of amyotrophic lateral sclerosis (ALS) is unknown. Several studies implicate both genetic and environmental risk factors, including metal toxicity or inadequate handling of toxins.

Objectives: The purpose of this study was to estimate metal exposure from assessed metal levels of hair samples obtained from volunteers with ALS and healthy controls. Due to the potentially neurotoxic effects of vanadium it was expected that endogenous levels in people with ALS would be significantly higher than those of healthy controls.

Methods: Neck region hair samples, 1 inch in length and consisting of at least 12 strands, were obtained from 100 ALS and 100 healthy control volunteers and analyzed for trace element content using neutron activation analysis (NAA). The International Atomic Energy Agency protocol for hair sample preparation was followed for cleansing the samples prior to NAA. Analysis of gamma peak results led to the ultimate part per million value used in statistical evaluations. Manganese, vanadium, aluminum, and magnesium levels in hair samples for both groups were measured. In addition, extensive epidemiological information was obtained from each volunteer, including disease and exposure histories.

Results: Vanadium and manganese levels were lower in volunteers with ALS compared to healthy controls. There was no difference between groups when comparing mean aluminum and magnesium levels.

Discussion and conclusions: We did not find significant differences in measured levels of vanadium, aluminum, or magnesium when comparing people with ALS to control volunteers. A potentially significant difference between manganese levels in healthy controls and ALS patients was

detected. Hair samples are a good measure of exposures during the period in which the hair was growing. Nevertheless, hair samples do not reflect exposures that may have occurred prior to this period. Manganese superoxide dismutase (SOD2) is known to play a role in anti-oxidant pathways. Very little is known, however, about the effects of low manganese levels on cellular function. Elucidation of the roles of subnormal vanadium and manganese levels in the body may provide insight into aberrant chemical pathways and potential modes of treatment. The data obtained in this study also defines baseline characteristics for hair metal analysis of healthy controls and ALS patients.

P251 ENVIRONMENTAL TOXICANTS AND GENE-ENVIRONMENT INTERACTIONS IN SPORADIC ALS

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Background: One hypothesis for the cause of sporadic ALS (SALS) is selective damage to motor neurons by an environmental toxicant. SALS appears to be increased in people exposed to heavy metals (1), solvents (1), agricultural chemicals (2) or living in rural areas (3). The increased incidence of SALS in airline pilots (4) and Gulf War veterans (5) may also be due to toxicant exposure. Gene-environment interactions related to toxicants may underlie susceptibility to this disease. Genetic defects in the pathways handling environmental toxicants may increase risk of ALS by reducing the capacity to respond to toxic insults.

Objectives: To investigate the effects and interactions between polymorphisms in detoxification genes and environmental exposure in SALS patients and controls. Genes examined were involved in the detoxification of heavy metals, agricultural chemicals and solvents. These were the metallothionein (MT) family, metal transcription factor 1 (MTF1), paraoxonase 1 (PON1) and glutathione synthetase (GSS).

Methods: Data on environmental exposures were collected using a self-reporting questionnaire. Polymorphisms were genotyped across the MT isoforms MT1A, MT1E, MT1F, MT1G, MT1H and MT2A, MTF1 and GSS in 186 SALS patients and 186 controls using Taqman® Genotyping Assays and sequencing. MT3 polymorphisms were screened in 87 SALS patients and 174 controls and PON1 polymorphisms in 143 SALS patients and 143 controls using SNaPshot® genotyping. Alleles, genotypes and haplotypes were compared between SALS patients and controls. Regression was used to test gene-environment interactions.

Results: *MT isoforms:* A polymorphism upstream of MT1E differed in SALS patients at the allele and genotype level. Common haplotypes across the MT1 isoforms also differed between groups. One MT3 haplotype interacted with exposure to metals to increase ALS risk. *MTF1:* A coding MTF1 polymorphism differed in the female SALS patients. *PON1:* A promoter polymorphism that lowered PON1 expression was associated with SALS. This polymorphism interacted with exposure to herbicides/pesticides at the allele level to increase the risk of ALS. Other promoter polymorphisms were associated with SALS at the genotype and haplotype levels. *GSS:* One GSS haplotype interacted with metal exposure and solvent/chemical exposure separately to increase the risk of SALS.

Discussion and conclusion: This work supports the hypothesis that polymorphisms in detoxification genes contribute to susceptibility to SALS in some individuals. In addition, changes in detoxification genes may act in combination with exposure to environmental toxicants to increase the risk of this disease.

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P252 IS DIABETES MELLITUS A PROTECTIVE FACTOR FOR ALS?

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Background: Few premorbid conditions have been identified which affect the progression of ALS or the probability of developing the disease. Glucose and insulin dysregulation occur in the disease; however, premorbid glucose and lipid dysregulation have not been previously studied as risk factors.

Objective: To determine the prevalence of Diabetes Mellitus (DM) in a large population of sporadic ALS patients and analyze the effect of this premorbid condition on disease outlook and presentation.

Methods: 1,704 sporadic ALS patients were tested for fasting blood sugar levels and interviewed for DM history. The prevalence of DM was compared to a US study (National Health and Nutrition Exam Surveys III) of 33,994 people. Disease outcome measures were compared between ALS patients with and without DM.

Results: Diabetes was found in 7.16% of ALS patients. The prevalence of DM in ALS patients over 60 (8.6%) was less than the prevalence found in the control population (19.3%). Diabetic patients had a later age of onset than non-diabetic patients by about 5 years

($p < 0.01$). When analyzed further, it was found that only male diabetics have a later age of onset ($p < 0.01$). No relationship was found between DM and gender, site of onset, or disease duration.

Conclusions: This study appears to have found a lower incidence of diabetes mellitus in ALS patients. It also showed that diabetic male patients typically had symptom onset 5 years later than non-diabetic male patients. These findings suggest that glucose and insulin dysfunction may significantly change the course of ALS.

P253 DIABETES MELLITUS IS UNDERREPRESENTED IN ALS AND NOT EXPLAINED BY BODY MASS INDEX

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Background: While caring for patients with ALS in Arkansas, we became suspicious that diabetes mellitus (DM) is underrepresented in this population. Prior studies have suggested ALS patients are leaner than controls, potentially explaining lower prevalence of DM.

Objective: To determine prevalence of DM and of being overweight or obese in patients with ALS compared to population controls and to determine if being overweight or obese influences disease progression.

Methods: Retrospective chart review of patients with sporadic possible, probable, or definite ALS (El Escorial criteria) seen in our clinic from 1998–2006. Patients self reported whether or not they had ever been told they had DM, and their weight prior to ALS symptoms. Prevalence of DM and being overweight/obese in our patients was compared to population controls using chi square analysis.

Results: Pre-ALS body mass index (BMI) was available for 226 ALS patients. 77/226 (34.1%) were normal/underweight (2 underweight) and 149/226 (65.9%) overweight/obese (60/226 or 26.5% obese). For those patients 50 and older, 55/167 (32.9%) were normal/underweight, 48/167 (28.7%) obese, and 112/167 (67.1%) were overweight/obese. In Arkansas, prevalence of being normal/underweight is 37%, obese 26%, and overweight/obese 63%. Therefore, our ALS patients were not leaner than population controls. DM was reported in 12/226 (5.3%) compared to population control prevalence rate of 6.5% (ns). For ALS patients 50 years and older, 12/167 (7.2%) had DM, compared to 17.1% for population controls 50 and older ($p = 0.00067$). Five patients had diet controlled DM, 7 took oral hypoglycemics, and none required insulin. Obese/overweight patients did not differ significantly compared to normal/underweight patients regarding time to become wheelchair bound or survival.

Conclusions and relevance: ALS patients 50 years and older were less likely to have DM compared to population controls. This could not be explained by pre-ALS BMI, since our ALS patients were not leaner than population controls. Why DM is underrepresented in ALS patients is unclear, but a potential explanation could have a genetic basis, with a susceptibility gene for DM closely linked to a gene that conveys neuroprotection.

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P254 AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND AUTOIMMUNE DISEASES (AID): CONCURRENCE IN AN INBRED ITALIAN FAMILY

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Background: Neuropsychiatric systemic lupus erythematosus may present as motor neuron disease clinically indistinguishable from ALS. The association between Amyotrophic Lateral Sclerosis (ALS) and Systemic Lupus Erythematosus (SLE) has been reported in literature only in three cases (1,2,3), two of which were classified as ALS-mimic syndrome(1,2).

Case report: A 45-years-old woman with Familial Systemic Lupus Erythematosus (SLE) treated with corticosteroids and hydroxychloroquine developed involuntary tongue movements diagnosed as myoclonus, followed six months later by a progressive bulbar and motor palsy typical of ALS. Neurological and neurophysiological examinations showed upper and lower motorneuron involvement both at the spinal and bulbar level. MRI of brain and spinal cord and CSF examination were normal. Electrophysiological studies (electromyography, transcranial magnetic stimulation) showed diffused signs of UMN and LMN impairment with normal nerve conduction and no conduction blocks.
No improvement of the neurological signs were observed after three months of treatment with cyclophosphamide followed by one intravenous immunoglobulin cycle and two plasmapheresis cycles. At present the patient shows a severe tetraparesis, the speech is not understandable and a feeding tube is necessary for nutrition. The patient belongs to an inbred family from a small Sardinian village with a history for both ALS and Autoimmune Diseases (AID). Her parents are consanguineous. A maternal second degree cousin developed the first signs of motor neuron disease at age of 58. He died at 60 with an ALS diagnosis. A maternal third degree cousin presented autoimmune polyendocrinopathy type III and ALS at age of 45.Genetic research for SOD1 mutations were negative both in the proband and in the still alive third degree cousin. In addition there are two sisters and two cousins affected by SLE, two maternal uncles with RA and one cousin with pemphigo, in whom no sign of MND has been found.

Discussion: The patient was finally diagnosed as familial ALS and familial SLE rather than ALS mimic syndrome because no evidence of concomitant cerebrovascular disease was found. Moreover no improvement with immunosuppressive therapy was verified as in ALS. This is, at the best of our knowledge, the first reported case of familial ALS (fulfilling the El Escorial revised Criteria) (4) associated with familial SLE. Although the role of autoimmune mechanisms in ALS pathogenesis is not clearly understood, the concurrence of ALS and AID in two subjects might suggest, in this family, a possible common pathogenetic mechanism.

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P255 AGGREGATION OF NEURODEGENERATIVE DISEASE IN IRISH ALS FAMILIES

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Background: Recent evidence of clinical overlap between ALS and fronto-temporal dementia suggests that ALS is part of a spectrum of neurodegenerative disease. If this is the case, it is likely that ALS and other forms of neurodegeneration share common susceptibilities, and that these susceptibilities may aggregate within kindreds.

Objective: To determine the degree to which neurodegenerative diseases aggregate in ALS kindreds using a population-based approach.

Methods: Using the Irish ALS register, all ALS patients diagnosed in the years 2005 and 2006 were asked to complete a detailed family history questionnaire. Ethnicity-matched controls were selected from spouses, and geographically matched individuals living in the patient's locality.

Results: To date, details of 70 ALS kindreds (2,093 individuals, (595 1st degree relatives)) and 42 control kindreds have been collected (1,158 individuals and 354 1st degree relatives).
An increased incidence of ALS in affected family kindred was detected. (λALS total=227.5, λ1o=557, λ2o=145, λ3o=93). Parkinson's Disease (PD) was also overrepresented in ALS kindreds compared with the control cohort (λPD total=9.2). The risk of Alzheimer's Disease (AD) appeared to be reduced in the ALS kindreds compared to the control population, (λDem total=0.74)
The overall risk of other neurodegenerative disease in ALS kindreds (excluding ALS) is high.(λrisk total=12.14).
The study demonstrated an unexpected increased frequency in first degree relatives of neuropsychiatric conditions Schizophrenia (n=3), manic depression (n=5) and suicide (n=3). These conditions clustered in kindreds with a strong family history of ALS/FTD, and were not reported in any of the 42 control kindreds.

Conclusions: Other neurodegenerative diseases occur more frequently in family members of patients with ALS. This observation supports the view of ALS as part of a larger continuum of neurodegenerative disease, susceptibility to which is likely to be genetically determined. Neuropsychiatric illness occurs more frequently in kindreds with ALS/FTD, and may represent an early manifestation of frontal dementia.

P256 ETHNIC VARIATION IN FREQUENCY AND CLINICAL FEATURES OF ALS: ANALYSIS OF A TERTIARY CLINIC-BASED COHORT IN HAVANA, CUBA

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Background: The incidence and prevalence of ALS may not be uniform across different ethnicities. Most population based studies have been conducted in Europe and North America, and have captured mainly those of European Caucasian origin. Although the lower incidence of ALS in non-European countries, and the lower incidence of ALS in African-Americans may have been confounded by reduced ascertainment, there is evolving evidence to suggest true differences may exist.
Full disease ascertainment of neurological disease is possible in Cuba, as medical care is available to all, and there is a well developed network of neurological care.
In a small preliminary study we have noted a reduced incidence of ALS in those who report their ethnicity as ''Black'' and ''Mulatto'' compared to those who report their ethnicity as ''White''.
Aim: To ascertain all cases of ALS diagnosed at the National Institute of Neurology at Havana over a 24 month period and to determine the incidence and clinical features of the disease within 3 defined groups – ''White'', ''Mulatto'' and ''Black''.

Methods: Approximately 50% of all Cuban patients are referred to the Institute for diagnosis of neurological disease. A study of all incident cases of ALS attending the Institute of Neurology and Neurosurgery at Havana commenced in October 2005. Clinical details, including self-reported ethnicity and family history, are collected from all patients and are included in the Cuban Register of ALS.

Results: Based on the known population structure in Cuba, findings from this clinic-based cohort suggest that the frequency of ALS is higher than expected in those of European origin, and lower that expected in those of mixed ethnicity. The mean age of disease onset is lower than that of European studies (50.1 years); and there is a male predominance of spinal-onset disease.

Conclusions: This is the first detailed clinic-based study of an ALS cohort from a country of mixed ethnicity. Our data suggests that ALS is commoner in those Cubans of European extraction compared to those of mixed ancestry. Further population-based analysis is underway.

P257 INVESTIGATION OF DIFFERENCE IN SUSCEPTIBILITY TO ALS IN CAUCASIAN AND AFRICAN-AMERICAN COHORTS

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Sporadic ALS (SALS) is a complex disorder of unknown etiology. Variations at multiple genetic loci interacting with each other and environmental exposures are hypothesized to modulate disease. Several laboratories including our own have studied functional candidate genes such as vascular endothelial growth factor (VEGF), the poliovirus receptor gene (PVR) and apolipoprotein E gene (APOE) to determine if variations in these genes are associated with SALS. Interestingly, PVR and APOE lie in a 300 kb region on Chromosome 19q13 which had earlier shown a positive LOD score of 1.96 in an affected relative pair (ARP) screen of non-SOD1 and non-linked ALS families (unpublished data). We therefore screened this region for possible association with sporadic ALS using family-based and case-control models with 34 SNPs (TaqMan assay) and localized an association signal within the *cbl-c* gene.
Mutations in the product of the *cbl-c* gene may affect degradation of tyrosine kinases in the motor neurons leading to motor neuron disease. The aim of this study was to investigate if a novel exonic polymorphism found in the *cbl-c* gene was associated with sporadic amyotrophic lateral sclerosis (SALS). Genomic DNA was isolated from a cell line of human leukocytes and screened using a four primer PCR reaction for the presence of a single cytosine insertion (insC) polymorphism between codons 418 and 419 of *cbl-c* that led to a frame-shift. Association between the presence of this polymorphism and sporadic ALS was investigated in Caucasian and African-American case-control cohorts (n=2,087 and n=56, respectively) using Chi-square analyses. The frequency of this polymorphism was significantly different between the two ethnic groups. No association was found in the Caucasian case-control cohort. In the African-American case-control cohort, however, we observed a trend for association in age-at-onset (AAO). Comparison of mean AAO revealed a 4.5 year difference between insC and wild type samples in the African-American group but due to sample size limitations our findings were inconclusive. We therefore conclude that the insC polymorphism found in the *cbl-c* gene is not associated with SALS in the Caucasian case-control cohort. However, a closer examination of this polymorphism needs to be carried out in a larger cohort of African-Americans to determine with greater certainty its AAO modifier effects.

P258 MOTOR NEURONE DISEASE AMONG MALTESE IN MALTA AND AUSTRALIA: MANY SPORADIC CASES ARE RELATED

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Background: Malta and the smaller island Gozo have excellent records of births, marriages and deaths, with details of consanguinity in the parish records. Many Maltese emigrated to Australia and their death records are available.

Objectives: To examine a population for evidence of kinship among sporadic cases of MND and to compare this with large samples of polios and control children.

Methods: Certificates of Maltese who died of MND were obtained from Malta and Australia. Birth certificates were used to find the parents. Details of the grand-parents and earlier forebears were found in the Public Registry and in the parishes. All marriages were checked for dispensations for consanguinity.
The medical records of 1,070 children with polio 1926–1964 were found and the parents, grand-parents and great grand-parents were traced: each polio was matched with a baptism control. Muscular dystrophy cases were followed in the same way. About 17,000 marriages were traced.

Results: There were 183 Maltese cases of MND. Six are still living with confirmed diagnoses. Forty five had emigrated to Australia, 2 had been born in the USA, and 1 lived in England. Fifteen had been born in Gozo, of whom 3 died in Australia. For Malta, the mean age of death was 65 yr for males (N=67) and 64 yr for females (N=45). For Gozo (N=14) it was 63 yr. For the 35 men and 10 women emigrants to Australia (mean domicile 30 yr), the mean age at death was 56 yr.
In only 3 kinship groups were there cases in 2 generations. Eighty cases had links to at least one other: 44% of the 183 cases were linked. There were groups with 12, 10, 9, 6 and 4 cases, 5 groups with 3 cases and 12 groups with 2 cases in each. Of the MNDs a greater proportion of parental and grand-parental marriages were consanguineous than those of the polios, controls and cases of muscular dystrophy.
There was an incidence of 2.1 MND /00 for Malta 1990–1999, but for New South Wales 1991–2006 for those born in Malta, the incidence was 2.7/00. The incidence of MND was higher in the more isolated parishes than in the larger, less isolated ones.

Discussion: The kinship of 44% of cases is greater than the 5–10 % of familial cases usually reported. This may be a feature of Malta or the methods of this study. Consanguinity may be far higher in Malta than in other cultures.

Conclusions: For Maltese, there is a very strong genetic component in sporadic cases of MND.

P259 NORTH-SOUTH GRADIENT OF ALS IN FRANCE: A MORE THAN TWO FOLD INCREASE IN SOUTHERN AREAS

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Background: ALS is a neurodegenerative disease described world wide with some focus areas such as Guam. Some studies have suggested a differential distribution of the cases according to latitude, the most recent one concerning Spain with an increased frequency in Northern areas.

Objectives: To describe the prevalence of ALS in France.

Methods: As no register exists in the country, we studied 2 parameters reflecting ALS frequency: 1) number of declared cases to the French Social Security (mandatory declaration as ALS is a disease with heavy social and medical burden); 2) number of patients taking riluzole (the only treatment labelled for ALS in France). These parameters were obtained for the years 2004 and 2005. Data were compared, when appropriate, to those from the corresponding ALS centers.

Results: Overall prevalence of ALS in France (per 100 000 inhabitants) was 4.78 and 4.86 for 2004 and 2005, respectively. When dividing the country in 4 areas (north, middle north, middle south, south), prevalence in 2004 was: 4.4 – 5.4 – 5.5 – 5.6; and in 2005: 4.3 – 5.4 – 5.8 – 6.3. Between the extreme northern region (Nord Pas de Calais) and the extreme southern region (Languedoc-Roussillon), prevalence rose from 3.48 to 7.6 in 2004 and 3.66 to 8.0 in 2005. When comparing recruitment of ALS centers in the country, such a gradient was already suggested by the ALS center report of 2006 in which 5/100 000 patients were followed in Nord Pas de Calais compared to 12/100 000 patients in Languedoc-Roussillon. As 1/3 of the followed patients are known to be of extra regional origin, the regional prevalence estimated from the recruitment of those 2 ALS centers was 3.4 vs 8. These data are very similar to the statistics issued from the administration inquiry.

Discussion and conclusions: A North-South gradient has been noted in France, stable between 2004 and 2005. It is, to our knowledge, the first time that such a highly significant gradient (2.35 fold increase) is described in an industrialized country. The reasons for such a gradient are difficult to precisely determine. However, an increased incidence of ALS has been described in Northern Spain. Because of common genetic background of some populations from Southern France and Northern Spain, we believe that this gradient is more likely explained by genetic factors than by environmental ones.

P260 MATERNAL AGE, SIBSHIP SIZE AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Early life exposure may play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS). Maternal age at delivery and sibship size have both been proposed to have an impact on the aetiology of a handful of diseases, especially those with a component of infection, and thus could affect ALS risk.

Objective: To estimate the associations between maternal age, sibship size and risk of ALS.

Methods: We conducted a nested case-control study based on the Swedish Multi-Generation Register between 1987 and 2005. In total, 768 ALS incident cases were identified from the Swedish In-patient Register and five controls per case were randomly selected by matching birth year and gender. Maternal age at delivery, birth order and number of younger siblings for the index persons were identified from the Multi-Generation Register. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) for ALS were estimated using conditional logistic regression.

Results: Both old ($\geqslant$41 years) and young ($\leqslant$20 years) maternal age at delivery were associated with increased risk of ALS (OR 1.7, 95%CI 1.1–2.5 and OR 1.5, 95%CI 1.1–2.1 respectively). Risk of ALS increased with increasing numbers of younger siblings, with an OR 1.3 (95%CI 1.0–1.8) for three or more younger siblings. The effect was modified by birth interval between index persons and their first younger siblings. Persons with more than one younger sibling, whose first younger sibling was born after 8 or more years, had the greatest risk of ALS (OR 2.8, 95%CI 1.5–5.3).

Discussion and conclusions: Both young and old maternal age at delivery were associated with higher risk of ALS among the offspring, compared with mothers aged 21 to 40, possibly due to different prenatal environments or environmental exposures before or after birth. Introduction of an infant into a household after 8 years without one was also associated with increased risk of ALS among the older siblings, possibly due to repeated exposure to active infections carried by the infant siblings.

P261 LONGITUDINAL CHANGES IN AGE AT ONSET AND SURVIVAL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN W PREFECTURE

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Background: It has been reported that the mean age at onset (AAO) of amyotrophic lateral sclerosis (ALS) has become older in recent years (1). However changes of AAO and survival according to year at onset (YAO) in ALS patients are not yet clarified.

Objective: This study investigated longitudinal changes in AAO and survival of ALS patients in W Prefecture.

Method: Questionnaires on ALS patients diagnosed using the El Escorial criteria were mailed to all clinics and hospitals in W Prefecture five times between 1 January 1998 and 31 December 2005. Clinical data at baseline for each patient including sex, date of birth, birthplace, AAO, site of initial symptoms, date of respiratory support applied, and date of death were collected to review at the end of follow-up. The List of Patients with Intractable Disease (certified by the Ministry of Health, Labour and Welfare of Japan) in W Prefecture was used to verify the data. The clinical duration in this series was determined from onset to earlier date of death or respiratory support applied. For longitudinal analysis of AAO, patients were classified into two groups by YAO, group A: onset in 2000 or thereafter and group B: onset before 2000. We used Kaplan-Meier survival curves and log-rank test for comparisons.

Results: There were 257 ALS patients (definite or probable) enrolled during the period examined. AAO in group A (mean$\pm$S.D; 67.2$\pm$9.4) was significantly higher than that in group B (61.6$\pm$11.1) ($p<0.0001$). Group B had significantly better prognosis compared to group A ($p<0.001$). Kaplan-Meier survival analysis showed that survival was significantly decreased with advancing AAO by 10-year age groups ($p=0.0039$), and also patients <65 years had significantly better survival than those $>=65$ years ($p=0.0001$). Bulbar palsy-onset was more frequent among patients over 70 years old than those below 69 years old ($p=0.017$). Patients with bulbar palsy-onset showed worse survival than those with lower extremity-onset.

Conclusion: It is suggested that ALS patients in W Prefecture who developed initial symptoms in 2000 or thereafter had older AAO, more frequently bulbar palsy-onset and shorter clinical duration than those who developed initial symptoms before 2000. Bulbar palsy-onset and AAO might be predictors of survival. The shift to an older mean AAO in recent years might be due to exogenous factors including changes in dietary patterns, vigorous physical labour, and unknown environmental factors in W Prefecture.

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P262 CLINICAL PHENOTYPES AND NATURAL HISTORY IN AMYOTROPHIC LATERAL SCLEROSIS VARIANTS

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Background: Understanding the biological basis of phenotypic variation in Amyotrophic Lateral Sclerosis (ALS) is increasingly important for genetic and therapeutic studies. Better understanding of clinical phenotypes can also provide important information on survival & prognosis relevant to patient care. Here we describe the characteristics of defined clinical phenotypes, as well as their natural history in relation to survival, in two large clinic populations acquired through analysis of case records at the King's MND Care and Research Centre London, UK and the Bethlehem MND Service in Melbourne, Australia.

Objectives: The main objective was to define the prognosis of clinically important phenotypic subgroups of people with ALS and related motor neurone disorders, including the flail arm and flail leg syndromes and PMA in relation to survival, and to understand related clinical features that might contribute to prognosis.

Methods: We used a common protocol with decision criteria for classifying phenotypes: ALS with bulbar, cervical and lumbosacral onset (Classical ALS); 'flail arm syndrome' (FA); 'flail leg syndrome' (FL) or Pseudopolyneuritic form of ALS; progressive muscular atrophy (PMA); and primary lateral sclerosis (PLS). The FA & FL phenotypes were distinguished from cervical onset & lumbosacral onset ALS respectively by the fact that they had predominantly lower motor neurone features and there was no functional involvement of a further region for at least 12 months after presentation. Patients with Kennedy's syndrome, SMA, MMN and other conditions not defined within the ALS/MND rubric were excluded ($n=210$).

The King's database analysed 430 patients seen between 1993–2007 and the Melbourne database contained data

on 389 patients. The censoring date was 1st May 2007. The two populations were analysed separately.

Results: At the time of analysis 203 patients in the Melbourne group and 235 patients from the King's group had died. Mean survival time (in months) of patients who had died at time of analysis showed that the FL phenotype had longest survival of 76.6 in King's and 75.5 in Melbourne. This was followed by FA phenotype, which was 58.2 (King's) & 57.8 (Melbourne). The survival of other ALS phenotypes were as follows: Bulbar onset 26.9 (King's) & 27.1 (Melbourne); Cervical onset 31.2 (King's) & 32.9 (Melbourne) and for Lumbosacral onset 36.9 (King's) and 35.7 (Melbourne).
Unpaired t-test to compare group mean survival times showed a highly significant difference between FL and Lumbosacral onset ALS (P value King's < 0.0002 & Melbourne < 0.0001) and FA and Cervical onset ALS (P value King's < 0.0001 and Melbourne < 0.0001).

Discussion and conclusions: This data from two independently collected & analysed populations shows remarkable similarities, and indicates that survival in the FL and FA differs significantly from ''Classical'' ALS phenotypes. Therefore, the FA & FL phenotypes are distinctive clinical and presumably biological variants of ALS.

P263 INCIDENCE OF MOTOR NEURON DISEASE AND SURVIVAL AFTER DIAGNOSIS: THE GENERAL PRACTICE RESEARCH DATABASE 1990–2005

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Background: Clinical series and population based studies have provided different estimates of the incidence of motor neuron disease (MND) and MND patients' survival. In clinical series, mean age of onset is under 60, with a median survival of 3 years, while population based studies have reported peak incidence occurring in the seventies and lower survival after diagnosis. Additionally, few studies have estimated incidence of MND among subjects over 80, which would be useful to determine whether MND is an aging-dependent (higher incidence with higher age) or age-dependent (highest incidence at a specific age) disease.

Objective: To estimate (i) the incidence of MND in a population-based study in the United Kingdom, and (ii) the survival of those incident cases.

Methods: The General Practice Research Database (GRPD) is a computerized database that contains clinical information, both clinical diagnoses and drug prescriptions, on more than 5 million Britons followed up by their general practitioners. We identified people with a first time computerized diagnoses of MND occurring from 1990 to 2005. To assess the validity of the MND diagnosis in the GPRD, we reviewed a random sample of 65 medical records of patients with a computer-based MND diagnosis. In our review, we were able to confirm 85% of the computerized diagnoses. Incident cases were followed up from date of diagnoses to death, last data collection or October 30, 2005, whichever came first. Median survival and its 95% confidence interval (CI) were computed using the Kaplan-Meier estimator. To estimate the association of age and sex with survival, we used a Cox proportional hazards model.

Results: During a total of 29,251,111 person-years of follow-up, there were 844 new diagnoses of MND (43% among women). Overall incidence of MND in this population was 2.9 cases per 100,000 person-years (2.5 among women, 3.3 among men). Incidence rates peaked for both sexes in the group 75–79 years old. Age-adjusted incidence, standardized to the European population, was 1.8 cases per 100,000 person-years among women and 2.9 among men. During 1,626 person-years of follow-up, 551 out of 844 MND patients died (65% of all patients). Median survival after diagnosis was 18.8 months (95% CI 16.5, 21.1). In the multivariate analysis, age but not sex was predictor of survival: subjects age 80 or older had more than four times increased mortality rate compared with subjects under age 50 (hazard ratio 4.4, 95% CI 3.0, 6.6).

Conclusion: These findings support the proposition that MND is an age-dependent disease, with incidence peaking before age 80 and decreasing afterwards, and higher incidence in men than in women. We observed a median survival from time of diagnosis of less than 2 years. Advanced age is the strongest independent predictor of worse prognosis.

P264 STUDY OF THE CIRCUMSTANCES AND CAUSES OF DEATH IN ALS

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Background: The different causes of death of patients with ALS have not been analyzed in a qualitative manner, in either published therapeutic assays or studies on prognostic factors. The reasons for this could be the difficulty in establishing with precision the exact causes of death in a population of ALS patients and a lack of confidence in the death certificates. A prospective study surveying the causes of death in ALS appears useful.

Objectives: To estimate the rate of mortality caused by ALS in 2005 in France using the information provided on the death certificates compiled by the ''Centre d'épidémiologie sur les causes médicales de décès'' (CépiDC). To prospectively survey the causes of death in victims of ALS from 1 March 2006 in 16 regions of France referenced by an ALS expert centre. To describe the circumstances of

death, the functional state of the patients at the moment of death and the morbidity associated with ALS.

Methods: A descriptive, prospective and multicentre study analyzing 302 deaths, undertaken over a minimum period of one year, covering all new deaths in patients followed in 16 ALS referenced centers in France. Three sources of information were exploited to analyze the circumstances and the causes of death: the referring neurology doctor from the ALS centre, the clinical patient file and the patient's attending physician if the death took place outside of a medical facility.

Results: To estimate the annual death rate from ALS, we exclusively used the information on the death certificates. For this, a request was made to ''CépiDC'' to receive all information concerning any deaths in France declared as ALS for the year 2005. 1200 patients have been declared with the diagnosis of ALS or motorneuron disease. 302 patients have been included (159 males- 143 females). 2.3% had familial ALS. Clinical form at onset was spinal (66.2%), bulbar (30.8%) and respiratory (2%). At time of death, according to El Escorial criteria, 81.5% had a definite ALS, 9.6% a probable and 5.3% a possible form. 33.1% had a non invasive ventilation and 3.3% had a tracheostomy. 35.4% were fed using a gastrostomy. Mean values of clinical evaluations were: MRC: 69.8 ± 43 (max: 150), ALSFRS: 16.7 ± 8.5, FVC: $47.5 \pm 22.1\%$, BMI: 21.6 ± 4 kg/m^2. 47.4% of patients died during sleep. 189 died in a medical facility (62.6%). The main causes of death were: terminal respiratory insufficiency (62.4%), inhalation pneumopathy (11.6%), pneumonia (5.3%), pulmonary embolism (3.2%) and undetermined (5%). Only 3 autopsies have been performed.
113 patients died outside of a medical facility (37.4%). The causes of death were terminal respiratory insuffisiency (46.9%), inhalation pneumopathy (6.2%), asphyxia (4.5%), pneumonia (1.3%), suicide (3.5%) and undetermined (18.6%). No autopsy has been performed.

Conclusion: A better understanding of the causes of death in people with ALS allows a better understanding of the natural history of the disease. Respiratory insufficiency is reported in more than 50% of deaths. 10 to 15% remained undetermined. About half patients died during sleep.