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SESSION 1 JOINT OPENING SESSION

C1 PATIENT SUPPORT IN JAPAN

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Background of ALS care in Japan: ALS has been considered as a most symbolic intractable disease in Japan. It is a cardinal subject of political grant governance both for basic science and on health and welfare research.

Patients with ALS prefer long-term admission to hospital rather than home care and 30 to 40% of patients request artificial ventilation at home or in hospital. Conditions for stable home care are unsatisfactory.

Problems to be solved: There is insufficient awareness of autonomy in each patient. Patients tend to depend upon long-term hospital admission rather than attempt independent life at home. However, there are insufficient hospital beds available for patients who request admission, especially in the case of long-term admissions to general hospitals.

Almost all prefectures have only a small number of neurologists and an insufficient number of home care nurses and caregivers. An increasing number of patients are living with ventilation under unsatisfactory conditions.

Strategy: 1) Increased availability of hospital beds for emergency use, respite for caregivers and occasional long-term admission; 2) Establishment of medical networks for the total management of ALS patients by neurologists and general practitioners with the keen cooperation of nurses, caregivers and other health and social care professionals; 3) Clinical pathways committing hospitals, care homes and patients' homes to stable long-term management of patients; 4) Construction of reasonable ward models in hospitals of the National Hospital Organisation and assessment of their effectiveness; 5) Promotion of autonomy, with the cooperation of JALSA and other patient support groups; 6) Mental and spiritual education through workshops and conferences; 7) Promotion of the Patients Support Bureau (PSB) project in all prefectures, focusing especially on the training of a coordinator.

Short term goals: With nationwide patient support, people with ALS will be able to select their way of life, whether at home or in a hospital or care home, from the earliest stages of the disease through to the final stage, and be able to maintain a meaningful personal life. They will be provided with sufficient palliative care without any bias on economic, legal or ethical grounds. Ultimately, all patients will be able to enjoy life with their families and maintain a higher quality of life.

C2 GUAM ALS: EPIDEMIOLOGICAL AND GENETIC OBSERVATIONS IN RELATION TO PDC AND DEMENTIA

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Background: ALS had an extremely high incidence among the Chamorros of Guam from 1945 to 1960. ALS is related to Parkinson-dementia complex (PDC), another endemic disease in Chamorros, and both show tau pathology of neurofibrillary tangles. The incidence of ALS declined over the next 40 years, as the environment and lifestyle of Guam became westernized. The rapid decline implicates an environmental factor, but specific environmental trigger(s) remain uncertain. Theories have included consumption of cycad products (directly, and possibly indirectly by eating fruit bats that are presumed to ingest cycad fruits) and low mineral concentration in the water supply. Genetic risk is implicated, because the population is inbred and went through a bottleneck. Asymptomatic Chamorros show an excess of tangles in cortical brain regions, suggesting that Chamorros are predisposed to form tau pathology.

Our research group studies the epidemiology and molecular genetics of ALS and PDC. We have also examined clinical and neuropathological aspects of late-life dementia among Chamorros.

Studies and results: The prevalence of ALS has continued to decline and currently is similar to that of sporadic ALS worldwide. PDC has declined but had a point prevalence of 1.5% in Chamorros aged 65 years and older in 2004. Late-life dementia (which we have termed Guam dementia) had a point prevalence of 12.4% in Chamorros of 65 years and older in 2004. Clinical features of late-life dementia resemble those of Alzheimer's Disease, but there appears to be prominent tau (tangle) pathology relative to amyloid pathology.

The APO-E e4 allele has a low frequency in Chamorros in general, and is not increased in ALS, PDC or Guam dementia. We previously found significant statistical association between a tau STRP and ALS/PDC. Ongoing genetic studies focus on tau, and a genome-wide linkage study of ALS/PDC.

Another research group reported that BMAA, a plant amino-acid found in cycads, occurs in high concentration in soluble and protein-associated pools in brains of patients who died from PDC. However, we have been unable to detect BMAA in frozen brain tissue from Chamorros with PDC.

Conclusions: 1) ALS, PDC and late-life dementia in Chamorros are a spectrum of disorders linked by tau pathology. 2) A gene-environment interaction may best

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explain the altered pattern of incidence of these disorders over time. 3) The tau gene may provide clues regarding genetic predisposition) 4. Ongoing studies of PDC and late-life dementia may help to clarify neuronal vulnerability and genetic factors, and may provide clues regarding the environment.

C3 OVERVIEW OF ALS VARIANTS IN THE KII PENINSULA OF JAPAN: EPIDEMIOLOGICAL, GENETIC AND CLINICOPATHOLOGICAL OBSERVATIONS IN RELATION TO ALS-PARKINSONISM-DEMENTIA COMPLEX

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Objective: To provide an overview and update on the epidemiology, genetics, clinical variations, and neuropathological findings of ALS and Parkinsonism-dementia complex (PDC) of the Kii peninsula of Japan.

Background: The southern coast area of the Kii peninsula is one of the highest incidence ALS foci in the western Pacific. The first description of Kii ALS was in an old book published in 1689 and pioneer neurologists in modern Japan paid attention to its high prevalence rates in this area. In 1950s Kimura and Yase found extremely high ALS prevalence rates in two villages, Hohara and Kozagawa. These high incidence ALS foci were reported to have disappeared in early 1980s. However, our recent survey disclosed not only continuing high incidence rates of ALS but also high incidence rates of PDC.

Materials and methods: We analysed clinical features and neuropathological findings of 12 autopsy cases. Epidemiological study on the five years' average incidence rates of major clinical phenotypes were investigated in approximately 80 registered cases whose onset of the disease had been between 1945 and 2003. Genetic analysis was performed in recent cases in which family history had been investigated precisely.

Results: 1) According to the clinical phenotypes, the 12 autopsy cases were subgrouped into four 'pure ALS' cases, four 'ALS with marked dementia' cases, two 'PDC' cases and two ''PDC followed by ALS'' cases. All phenotypes showed the common neuropathological findings of a combination of ALS pathology and neurofibrillar tangles (NFTs) without senile plaques. Numerous NFTs were present in all cases of ALS with dementia, PDC and PDC followed by dementia, while NFTs of pure ALS cases varied from a few to numerous although the pattern of NFT distribution was similar to PDC. These findings suggest that pure ALS, ALS with dementia and PDC with or without ALS are on a spectrum of a single entity of ALS-Parkinsonism-dementia complex. 2) Descriptive epidemiology showed mild decline of the classic ALS phenotype, mild increase of the ALS with dementia phenotype and marked increase of the PDC phenotype. Low incidence rates of the PDC phenotype before 1990 may be due to the fact that the surveys before 1990 had been focused on ALS and little attention had been paid to PDC. 3) Genetic studies disclosed that family history was positive for ALS/PDC in approximately 80% of the cases although the pattern of transmission did not show Mendelian inheritance. We found 10 patients who developed ALS or PDC 24 to 50 years after having moved away from the village. Family history was positive for ALS/PDC in all of them.

Conclusion: ALS in the Kii peninsula is one of the clinical phenotypes of an entity of ALS-Parkinsonism-dementia complex that is neuropathologically characterized by a combination of ALS pathology and many NFTs. High incidence rates seem to have continued over the 50 years we studied, and the rates of familial occurrence are high. These findings suggest that genetic predisposition is important in the pathogenesis of ALS-Parkinsonism-dementia complex in the Kii peninsula.

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SESSION 2A NEUROGENOMICS

C4 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 coregulatory 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 EIG 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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C5 GENE EXPRESSION PROFILE OF SPINAL MOTOR NEURONS IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: The causative pathomechanism of sporadic amyotrophic lateral sclerosis (SALS) is not clearly understood. There have been extensive studies using animal models and culture systems for familial ALS, especially with SOD1 mutations, but no similar approach is available for studying SALS. Microarray analysis is one of the appropriate approaches to understand the pathological pathway related to the neuronal degeneration process in sporadic neurological disorders. However, in the lesions of SALS spinal cords, there are reduced numbers of motor neurons with glial cell proliferation, making it difficult to examine motor neuron-specific gene expression by conventional microarray analysis.

Objectives: For the purpose of elucidating the pathogenesis of SALS, we reveal motor neuron-specific gene expression profile in SALS using microarray technology combined with laser-captured microdissection (LCM).

Methods: Fresh specimens of lumbar spinal cord from 14 SALS patients and 13 neurologically normal patients were obtained at autopsy. The pulsed laser microbeam cut precisely around the targeted motor neurons in the spinal ventral horn. RNA was extracted from LCM-isolated cells and reverse transcription and T7 RNA polymerase amplification of RNA were performed prior to DNA microarray analysis. RNA was extracted as well from the total homogenates of ventral horn gray matter of spinal cords. The data for each differential gene expression level obtained from microarray analysis (BD Atlas Glass Microarray System: Clontech) was reconfirmed by real time reverse transcription polymerase chain reaction and in situ hybridization.

Results: Spinal motor neurons showed a distinct gene expression profile from the whole spinal ventral horn. Three percent of genes examined were down-regulated, and 1% were up-regulated in motor neurons. Down-regulated genes included those associated with cytoskeletal/axonal transport, transcription, and cell surface antigens/receptors, such as dynactin, microtubule-associated proteins, and early growth response 3 (EGR3). In contrast, cell death-associated genes were mostly up-regulated. Promoters for cell death pathway, death receptor 5, cyclins A1 and C, and caspases-1, -3, and -9, were up-regulated, whereas cell death inhibitors, acetyl-CoA transporter, and NF-κB were also up-regulated. Moreover, neuroprotective neurotrophic factors such as ciliary neurotrophic factor (CNTF), hepatocyte growth factor (HGF), and glial cell line-derived neurotrophic factor were up-regulated. Inflammation-related genes, such as those belonging to the cytokine family, were not, however, significantly up-regulated.

Discussion and conclusions: Microarray analysis on the laser-captured motor neurons provided us with significant information about motor neuron degeneration and dysfunction in SALS. Such information cannot be obtained by whole spinal cord tissue microarray assay. In addition, we are now analyzing the sequential motor neuron-specific gene expression in terms of motor neuron degeneration process, which will provide an avenue for new molecular targeted therapy for SALS through developing the animal or cell models mimicking these molecular events determined in human SALS patients.

C6 GENE EXPRESSION PROFILE OF SPINAL MOTOR NEURONS IN THE G93A SOD1 MOUSE MODEL OF ALS AT DIFFERENT TIME POINTS IN THE DISEASE COURSE

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Background: Amyotrophic lateral sclerosis (ALS) is one of the most common adult onset neurodegenerative diseases, and while 90% of the cases are sporadic (SALS), 10% are familial (FALS). The most common cause of FALS is mutation of the SOD1 gene. Transgenic mice carrying mutant forms of SOD1 develop a neuromuscular disease very similar to human ALS in both phenotype and histopathological hallmarks.

Objectives: 1) To investigate changes in gene expression profiles of degenerating spinal motor neurons (MN) isolated from human G93A SOD1 mice, human WT SOD1 mice and non transgenic littermates (LM) at different stages of the disease (60, 90 and 120 days); 2) To identify pathways involved in the development of the neurodegenerative process.

Methods: Approximately 1000 motor neurons have been isolated from the lumbar spinal cord of each animal. RNA was extracted using Picopure kit (Arcturus), amplified using the RiboAmp Amplification kit (Arcturus) and labelled using the BioArray High Yield RNA Transcript Labelling Kit (Enzo). 10 μg cRNA was applied to the Affy MOE430A GeneChip, and data analysis was performed using ArrayAssist (Iobion).

Results: At 60 days, 258 genes were differentially expressed when comparing G93A SOD1 mutant mice with their non-transgenic LM. The G93A transgenic mice show a significant increase in both transcriptional and translational functions. Significant increases occur in the expression of genes relating to carbohydrate metabolism, the electron transport chain and three subunits of ATP synthase. At 90 days, transcripts involved in carbohydrate metabolism are still up-regulated, while genes involved in transcription and mRNA processing are down-regulated. At 120 days, the transcription profile of the G93A SOD1 mutant mice shows a significant change in 167 genes. At this late disease stage, the transgenic mice show a marked degree of transcriptional repression, involving key genes, e.g. NDN, TAF9 and RNA polymerase 1-1, while many cyclins regulating the first steps of the cell cycle, e.g. cyclin L1, E2 and D2 show increased expression. Interestingly, given the hypothesis that oxidative stress may play a role in ALS, genes involved in antioxidant activity and stress response are significantly decreased, underlining a deficit in this important cellular defence. No significant alterations in gene expression have been found comparing WT SOD1 mice and their littermate controls.

Discussion and conclusions: The up-regulation of genes involved in the transcription and translation processes as well as in carbohydrate metabolism suggests the activation of a strong cellular adaptive response in the first stage of the disease. At the 120-day time point, consistent with results obtained by the analysis of NSC34 cell line transfected with vector expressing human G93A SOD1 (1), the transgenic mice present a strong impairment in transcriptional function. Another interesting aspect of this stage is the up-regulation of the cyclin family. This result suggests that what might be occurring in ALS is the same mechanism found in other neurological pathologies (2,3), with unsuccessful re-entry into the cell cycle and consequent cell death.

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C7 WHOLE GENOME MICROARRAY ANALYSIS OF MOTOR NEURON VULNERABILITY IN G93A-SOD1 AND P301L-TAU TRANSGENIC MOUSE MODELS OF ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that selectively affects motor neurons. A small subset of familial ALS patients carries a mutation of the copper/zinc superoxide dismutase 1 gene (SOD1). Another subset of patients with ALS suffers from familial frontotemporal dementia with ALS, caused by mutations of tau. Transgenic animal models expressing SOD1 have been extensively studied and factors that may contribute to motor neuron degeneration have been identified, such as disruption of axonal transport, glutamate metabolism, oxidative stress, copper metabolism, and growth factors. However, how these and other yet unknown factors cause motor neuron degeneration in sporadic ALS remains unknown. Studying motor neuron degeneration in a transgenic animal model expressing mutant protein tau linked to frontotemporal dementia with ALS in addition to SOD1 mice may help to close this crucial gap in knowledge.

Objective: We aimed to characterize the molecular events involved in the initiation of motor neuron degeneration that may be relevant in both familial ALS linked to SOD1 mutations and FTD with ALS linked to TAU-P301L. We compared early motor neuron-specific gene expression in two mouse models of ALS before the onset of any known pathological changes in the spinal cord. Mutant SOD1 (G93A-SOD1) and mutant tau (P301L-TAU) mice were studied to identify overlapping differentially expressed genes. The expected gene set may elucidate the cause of motor neuron degeneration and may also function as biomarkers for the disease, potentially aiding earlier diagnosis of ALS.

Methods: DNA microarray technology in combination with laser-capture microdissection (LCM) was used to detect gene expression changes at the early stages of the disease prior to neurodegeneration. Spinal cords were dissected from 3-month-old female transgenic mice and their non-transgenic littermates. Axial cryostat sections from lumbar spinal cords were fixed in ethanol and stained with Cresyl violet. Motor neurons from the ventral horns were microdissected on a PixCell Arcturus LCM instrument. RNA extracted from the collected motor neurons was subjected to microarray experiments using Agilent's Mouse Whole Genome Oligonucleotide Microarray featuring 45,000 gene probes.

Results and discussion: We generated a list of differentially expressed genes for each mouse strain and identified overlapping and genotype specific sets of genes. The overlapping genes are likely to be relevant in the etiology of motor neuron degeneration independent of its genetic cause. Selected gene expression changes will be confirmed using in situ hybridization and RT-PCR. Their relevance to ALS will be confirmed in functional studies using animal models and post-mortem ALS patient samples.

C8 MOLECULAR PATHWAY ANALYSIS OF AMYOTROPHIC LATERAL SCLEROSIS BY GENOME-WIDE EXPRESSION PROFILING OF HUMAN BLOOD

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons, the etiology and pathogenesis of which still remain unknown. Incidence rates for ALS in Europe and North America range between 1.47 and 2.7 per 100,000/year. Life expectancy after the start of clinical symptoms is about two to five years, where respiratory insufficiency is a major problem in the end-stage of the disease. In approximately 10% of patients with ALS, a mostly autosomal dominant mode of inheritance is observed. The diagnosis of ALS is based on clinical features, electrophysiology and exclusion of ALS mimics, which usually takes from six to 12 months.

With regard to diagnosis or disease progression in ALS, neither a definitive diagnostic test nor surrogate marker is available. Recently, proteomic profiling of biofluids by mass spectrometry identified protein species with altered levels in ALS patients. It is hypothesized that blood genomic fingerprinting may be a way to find candidate markers.

Objectives: By using expression profiling we looked at the possibility of using blood as a surrogate tissue in the diagnostic phase and looked for candidate genes involving pathogenesis and disease progression.

Methods: Specific expression profiles of whole blood from ALS patients and healthy donors were compared using oligo-array and Illumina Sentrix HumanRef-8 Expression BeadChip in order to find marker genes and to find novel pathways that influence disease severity and progression.

Results: Results of the genome-wide expression profiling will be presented. Part of this analysis consists of the combination of profiles of both platforms. Interim analysis of the expression profiles of 19 patients versus 19 healthy controls with oligo-array method results in significant differential expression of 74 genes. Patients' genes tend to cluster together. Their function varies from regulation of transcription, cellular transport and protein activation. The function of 20 genes is still unknown. Clinically interesting are genes involved in apoptosis, genes with axonogenese activity and a gene containing the copper binding domain of SOD1.

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SESSION 2B DISEASE HETEROGENEITY

C9 MONOMELIC MOTOR NEURON DISEASE IN EUROPE: PROGRESSIVE AND NON-PROGRESSIVE FORMS

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Background: There are many reports of patients with motor neuron disease (MND) with single limb involvement, including some in Western countries. In Asia, Hirayama disease has been separately defined as a distinct entity. Generally, monomelic MND is a benign condition with no clinical progression over time. No particular initial signs have been described that might indicate a risk of progression when a patient is first observed.

Objectives: To describe a population of 17 patients regularly followed in order to ascertain findings which could imply a progressive disorder.

Methods: A group of 17 patients (14 males, aged from 25 to 60 years at disease onset) were selected, in accordance with the following inclusion criteria: presentation with painless and insidious onset of focal weakness and wasting of one limb, of neurogenic cause; a history of progression over a period of at least three years without clinical involvement of any other limb or body region; pure motor features without sensory symptoms or signs; no other disease and no familial history; normal neuroimaging and somatosensory evoked potentials; normal CSF and blood investigations. After inclusion, the patients were followed for a minimum of three years (three to eight years) and underwent periodic clinical and neurophysiological assessment (EMG and transcranial magnetic stimulation – TMS).

Results: There were two clinical syndromes at presentation, an upper limb (seven patients) and a lower limb disorder. Four patients showed clinical and neurophysiological progression to other regions. In two cases more severe weakness developed in the arm muscles initially affected, with progression to more proximal muscles, opposite limb, and lower limb and neck muscles. In two cases progression evolved to homologous segments in the opposite leg and proximal muscles of the first affected leg. In the other cases there was no clinical or neurophysiological progression. Progression was a feature only of those patients who presented with distal weakness, whether in the upper or lower limb. Those presenting with proximal weakness never showed progression after the initial period of development of the syndrome. One patient developed multifocal conduction block, responsive to immunotherapy. Brisk reflexes in wasted muscles ($p=0.32$) or EMG abnormality in the opposite limb at presentation were of no prognostic value in predicting the later development of clinical progression ($p=0.67$). On the other hand, abnormal TMS at presentation was a useful predictor of subsequent progression ($p<0.019$).

Conclusion: Patients with monomelic MND affecting proximal segments have a benign course. Patients with distal weakness may slowly experience progression to other regions. Abnormal TMS can identify upper motor neuron involvement and suggest potential progression (ALS patients with slow progression).

C10 THE INFLUENCE OF RARE AMYOTROPHIC LATERAL SCLEROSIS (ALS) PHENOTYPES ON SURVIVAL

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Background: ALS clinical heterogeneity is known and can be confusing. There may be predominance of the peripheral or central signs of motor neuron involvement, or a predominant initial involvement of one region: bulbar (progressive bulbar palsy), upper limbs ('flail arm' syndrome, FA), lower limbs ('flail leg' syndrome, FL), one limb (monomelic amyotrophy) and unilateral limbs (hemiplegic type). In addition, a number of epidemiological studies have reported clinical, molecular and geographical ALS risk factors and these results also lack uniformity. In this context, the recognition of ALS subforms may have significance for ALS differential diagnosis, prognosis and survival.

Objective: To estimate the occurrence of FA and FL phenotypes and their influence on survival in ALS individuals.

Methods: We carried out a retrospective study on 288 sporadic ALS patients seen at the Krakow MND Centre between January 2002 and January 2006. All participants were classified according to El Escorial Criteria. From ALS patients, FA and FL individuals were selected. FA was defined as a predominantly proximal LMN of the upper limbs in a more or less symmetric manner, without significant functional involvement of other regions at clinical presentation. FL was defined as a predominantly distal LMN of the lower limbs, without significant functional involvement of other regions at clinical presentation. The patients' demographic characteristics were compared between groups.

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Results: FA phenotype (n=19) is more common than FL (n=14) in ALS patients studied. There is predominance of males. The male to female ratio was, in FA, 5.3:1, FL, 3.6:1 and in remaining ALS cases 1.5:1, respectively.

There was a difference between the mean duration of the disease in studied groups: FA, 74.2, FL, 53.1, limb onset ALS, 58.2, bulbar onset ALS, 42.1 months, respectively. During the time of observation four FA and three FL individuals died compared to 33 out of 288 individuals who died from the remaining ALS patients. The mean survival of those FA patients that had died was: 34.5, FL: 29, bulbar onset ALS: 28.7 and limb onset ALS: 43.7 months, respectively.

Detailed analysis of progression of the disease in FA and FL individuals demonstrated that the location of the second sign (i.e. in the trunk in FA cases, proximally in the upper limbs in FL cases) was associated with shorter survival because of accompanying progressive respiratory failure.

Conclusions: FA and FL phenotypes occurred in less than 10% of ALS patients, with predominance of males. The mean duration of ALS is usually longer in FA and FL individuals compared to classic ALS cases. However, the survival in FA and FL cases could be dramatically shortened when second signs of disease have occurred in a specific location. What determines this factor is unknown and requires further study, including larger groups of patients.

C11 ALS AT THE ONSET: TOPOGRAPHY OF UPPER AND LOWER MOTOR NEURON WEAKNESS

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Background: Weakness in ALS is characteristically focal and contiguous early in the disease. Its upper motor neuron (UMN) and lower motor neuron (LMN) components are poorly characterized.

Objective: To separately characterize UMN and LMN components of weakness early after the onset of ALS.

Methods: We undertook a retrospective cross-sectional chart review of 100 ALS patients seen early in their course. We quantified severities of UMN and LMN abnormalities for all body regions with a simple ordinal scale. We indexed these to body region of first symptoms and analysed relative to this region. We evaluated indices of relative and absolute severities and onset progression rates. We correlated findings with survival data, available in 73% of patients.

Results: Mean age was 62 years (range 26–85 years). Fifty-eight per cent of patients were men and 42% were women. Ninety-two per cent had sporadic ALS and 8% had familial ALS. The average duration of symptoms prior to evaluation was 11 months (range 3–36 months).

Weakness was focal and contiguous in 98% of patients. Virtually any region of the body could be abnormal: 29% had bulbar onset; 34% had arm onset – right arm in 24% and left arm in 10%; 6% had truncal or respiratory onset; and 29% had leg onset – right leg in 13%, left leg in 15%, and both in 1%. Motor abnormalities were diffuse or of uncertain origination in the remaining 2%.

UMN and LMN components of the weakness were severest in the index regions and decreased radially away from these regions. Contiguity of body regions for UMN and LMN levels was similar except in the arms, where contiguity at UMN level was with the ipsilateral leg, but contiguity at LMN level was with the contralateral arm (consistent with somatotopic anatomy).

Both UMN and LMN weakness preferentially spread to caudal regions over rostral regions. Severity of UMN and LMN involvement varied widely and varied independently of each other. Onset progression rates were established early, varied widely, and correlated with survival. Patients with highest progression rates had a 50-50 mix of UMN/LMN involvement but those with 50-50 mix did not necessarily have high progression rates. Onset progression rates were higher in patients with bulbar and arm onset than in patients with truncal or respiratory and leg onset. Patients with truncal or respiratory onset had the shortest survival even though they had the slowest progression rates.

Discussion and conclusions: These features suggest motor neuron degeneration in ALS: 1) is a focal process for both UMN and LMN; 2) advances contiguously and independently for both UMN and LMN and creates graded abnormalities radially away from their respective onset foci; 3) has variable rates of progression that are established early; and 4) summates at the UMN and LMN levels as the disease progresses until it ultimately involves the respiratory system, at which time it appears to be diffuse.

C12 MND/DEMENTIA IS A UNIQUE NOSOLOGICAL ENTITY AND NOT JUST AN OVERLAP BETWEEN MND AND FRONTOTEMPORAL DEMENTIA

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Background: The occurrence of cognitive and behavioural symptoms reminiscent of the fronto-temporal dementia (FTD) in a subgroup of patients with motor neuron disease (MND) has been increasingly recognized in recent years. However, it remains unclear whether motor neuron disease/dementia syndrome (MND/D) constitutes simply an unusually frequent combination of MND and FTD, or a separate disease entity with its own characteristics. Moreover, many studies do not separate symptoms characteristic for the three subgroups of FTD: the frontal variant (fv), non-fluent-progressive aphasia (NFPA) and sementic dementia (SD).

Objectives: To determine the specific behavioural, cognitive and pathological features of MND/D and compare them with the typical features of the three subgroups of FTD.

Method: We followed up prospectively 15 patients with the clinical MND/D syndrome. In 10 patients we were able to perform a pathological examination of the brain. We compared the clinical and pathological results with those of patients belonging to all three FTD subtypes.

Results: The pattern of behavioural and cognitive changes in MND/D was in many aspects different from that of FTD. The psychiatric picture was characterized by early florid psychotic prodromal stage with delusions and hallucinations, although such symptoms can occur in fvFTD. Their severity and frequency would be unusual, particularly at an early stage. Another consistent feature was a severe progressive non-fluent aphasia with a consistent impairment in word and action processing. Again, the clinical picture does show some similarities to NFPA, but also significant differences. Pathologically, all examined MND/dementia cases were associated with ubiquitin-positive inclusions. Such inclusions were also observed in some FTD patients, but the pathological changes in this group were much more heterogeneous.

Discussion and conclusions: Both in terms of the clinical presentation and pathology MND/D constitutes a remarkably homogenous group, characterized by frequent occurrence of psychosis and aphasia as well as the consistent presence of ubiquitin-positive inclusions. It shares some features with FTD, particularly with the fvFTD and NFPA. In contrast, we have never observed a combination of SD and MND. We postulate, therefore, that MND/dementia should be considered as a separate entity within the spectrum of the 'Pick complex'.

C13 MOTOR FINDINGS PREDOMINATE IN FRONTOTEMPORAL DEMENTIA

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Objective: To determine the extent to which Parkinsonism and motor neuron disease are present, clinically and pathologically, in patients with dementia.

Background: Frontotemporal dementia (FTD) is a progressive dementia condition characterized by selective degeneration of the frontal and anterior temporal lobes, whereas ALS is a motor neuron disease with progressive loss of upper and lower motor neurons. Because these diseases attack different parts of the brain, the specialists caring for patients with the two conditions have vastly different areas of expertise and interests. However, recent reports suggest that the two disorders often develop together in the same patients. Motor findings in dementia may have different diagnostic utility, but the prevalence of specific motor features in dementia subtypes has not been studied prospectively.

Methods: Prospectively, 107 patients with dementia and 11 healthy controls underwent a detailed neurological examination and electromyography (EMG) of four limbs and tongue. All patients were enrolled in an autopsy programme and 15 of the patients studied had autopsies performed. A speech pathologist performed a clinical assessment with tongue strength and swallowing measures using fiberoptic endoscopic evaluation of swallowing (FEES) in a subset of patients. Clinical diagnoses included frontotemporal dementia known to have MND at the time of referral (FTD-MND, $n=7$), FTD ($n=37$), progressive supranuclear palsy (PSP, $n=8$), progressive non-fluent aphasia (PA, $n=7$), corticobasal degeneration (CBD, $n=14$), semantic dementia (SD=15), and Alzheimer's disease (AD, $n=19$). El Escorial criteria for an ALS diagnosis were used.

Results: Of the 37 FTD patients, 11 had abnormal EMG studies and clinically one had bulbar onset MND. Five had new diagnoses of ALS based on the EMG study and six had more subtle EMG abnormalities concerning MND. In one patient there was a diagnosis of a non-specific myopathy. Of the seven PA patients, just one had an abnormal EMG and met criteria for a diagnosis of ALS. Of the 15 SD patients, one had EMG abnormalities in one limb which will be followed over time to look for development of MND. Among the 19 AD patients, 8 PSP patients, 14 CBD patients, and 11 controls, only one patient with an atypical form of AD had EMG abnormalities in one limb. Parkinsonism and/or supranuclear gaze palsy was present in all CBD/PSP, six PA, and two FTD-MND patients, but few FTD, AD or SD patients. Swallowing abnormalities were present to some degree in all groups and were not predictive of motor neuron disease. Similarly, there have been no autopsy cases in this cohort of MND associated with any form of dementia other than FTD. Cases with associated motor neuron disease have ubiquitin-positive, tau-negative pathology.

Conclusions: The prevalence of motor neuron abnormalities in patients with FTD may exceed 30% at the time of presentation, which significantly impacts predicted survival. Owing to the low rates of MND in other dementia subtypes, clinical or EMG findings that suggest MND should heighten suspicion for FTD. Swallowing abnormalities are not specific for MND, and EMG is necessary to determine the true etiology.

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C14 BEHAVIOURAL CHANGE IN PATIENTS WITH AND WITHOUT COGNITIVE IMPAIRMENT IN ALS

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Background: Frontal lobe pathology can cause either cognitive or behavioural impairments, and profound behavioural change is the hallmark of frontotemporal dementia (FTD). While frontotemporal pathology occurs in ALS, little is known about the significance of behavioural changes in this population.

Objective: To examine and compare the frequency, type and degree of behavioural changes in cognitively impaired and cognitively normal patients with ALS.

Methods: We initially evaluated 47 patients with ALS for the presence of cognitive impairment. Patients with scores 1.5 standard deviations below the mean for age and education-matched norms on two or more of the following measures were classified as cognitively impaired: Trail Making Test, Verbal Fluency, Design Fluency, Color-Word Interference, Digit Span, and Mental Control. Behavioural changes were assessed using the Frontal Systems Behaviour Scale (FrSBe), a questionnaire completed by a caregiver. We compared premorbid with current behaviour to define a change score. New onset behavioural impairment was defined as a 2-standard deviation (SD) change from premorbid levels on either a FrSBe subscale (Apathy, Executive Dysfunction or Disinhibition) or the Total score. Patients with significant head injury, pre-existing dementia or major depression were excluded.

Results: Twenty-three patients were cognitively impaired (CI) and 24 were cognitively normal (CN). The groups did not differ demographically or in relation to disease factors (FVC, ALSFRS scores, site of onset, or duration of symptoms). Behavioural change associated with the onset of ALS was reported in 56% of CI and in 58% of CN. Apathy was the most prevalent abnormality in both groups. The CI groups showed a mean increase in apathy of 3 SD versus 2 SD for CN patients. The CI group demonstrated, on average, a 1.5 SD increase in executive dysfunction, with no change in the CN group. Neither group revealed significant changes in disinhibition. Total scores increased by 2 SD in the CI group but only 1 SD in the CN group. Scores on the executive dysfunction scale trended to correlate with cognitive impairment ($p=0.052$) but apathy, despite the high prevalence, did not.

Discussions and conclusions: Caregivers commonly reported behavioural changes, particularly apathy, whether or not patients were cognitively impaired or cognitively normal. However, greater behavioural change occurred in patients with cognitive deficits, suggesting that some of the observed change relates to the ALS-cognitive syndrome. Treatable factors such as hypoxia, sleep disturbance, and medication may also contribute to the high frequency of behavioural change. Additionally, standard behavioural questionnaires may not adequately control for disease-specific factors such as motor weakness which may be misinterpreted by caregivers as apathy. These issues are worthy of further study.

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SESSION 3A LESSONS FROM OTHER MOTOR NEURON DISORDERS

C15 RESPONSE OF MICROGLIA IN A MODEL OF MOTOR NEURON PATHOLOGY

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Microglia, one type of glial cells in the CNS, are initially considered to play significant roles as immunocompetent and scavenger cells. Microglia exhibit ramified morphology in normal brain; they are activated under pathological conditions and show a number of features including morphological change, proliferation, migration and induction of various biologically active molecules. Increasing numbers of reports indicate that activated microglia initiate or facilitate inflammatory and degenerative processes by producing neurotoxic substances. Likewise, a neuroprotective role of microglia has also been suggested from studies showing the production of a variety of neurotrophic factors.

Axotomized facial motor nucleus is an advantageous *in vivo* model to study the functional roles of microglia on injured motor neurons in brain pathology. Transection of the facial nerve causes activation of microglia and stimulates the proliferation. The activated microglia migrate toward the injured motor neurons and wrap up the cell bodies. A number of studies from our laboratory strongly suggested that the activated microglia surrounding motor neurons following facial nerve axotomy play neuroprotective roles by secreting neurotrophic factors such as BDNF and GDNF, and also by increasing expression of glial glutamate transporter, GLT-1. However, in order to function properly at the site of the lesion, microglia must migrate close to the injured motor neurons. Thus, cell migration is an important first stage of microglial response to ameliorate the damage to motor neurons.

We have recently shown that extracellular ATP induced membrane ruffling and chemotaxis of microglia and suggested that the effects are mediated by Gi/o-protein-coupled P2Y12 receptor (P2Y12R). We showed here that the ATP-induced chemotaxis of microglia is also regulated by the ionotropic receptor, P2X4R in addition to the P2Y12R. Stimulation of G-protein-coupled receptors leads to activation of phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K). We examined the effect of PLC and PI3K inhibitors on the formation of membrane ruffling and the chemotaxis of microglia following the stimulation by ATP. A PLC inhibitor inhibited both membrane ruffling and chemotaxis, while PI3K inhibitors suppressed only chemotaxis without inhibiting the membrane ruffling. Phosphorylation of Akt, which is known to be a downstream target for PI3K, was enhanced by ATP stimulation. The increase in Akt phosphorylation was suppressed by chelating extracellular calcium. These results indicate that activation of PI3K pathway is modulated by the extracellular calcium influx suggesting a possibility that ionotropic P2XRs are involved in the PI3K activation. Studies by using various antagonists and short-hairpin RNAs against P2XRs showed that suppression of P2X4R reduced the ATP-induced chemotaxis of the cells.

These results indicate that P2X4R, in addition to P2Y12R, is involved in the ATP-induced chemotaxis of microglia.

C16 MOLECULAR-TARGETED THERAPEUTICS FOR SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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Spinal and bulbar muscular atrophy (SBMA) is a motor neuron disease characterized by slowly progressive muscle weakness and atrophy of bulbar, facial and limb muscles. The onset of weakness is usually between 30 and 60 years followed by a slow progression of neuromuscular symptoms. Bulbar palsy often aggravates in the advanced stage of this disease, resulting in life-threatening respiratory tract infections and eventual early death. To date, no curative therapy has been established for SBMA.

The cause of SBMA is expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, in the first exon of the androgen receptor (AR) gene. SBMA chiefly occurs in adult males, whereas neurological symptoms are rarely detected in females having a mutant AR gene. To elucidate the sex-dependent neurological phenotypes of SBMA, we generated a transgenic mouse model carrying full-length AR containing 97 CAGs driven by a chicken ß-actin promoter. The male transgenic mice exhibited marked progressive motor impairment and nuclear accumulation of mutant AR, but neurological phenotypes were not observed or were far less severe in the females. Leuprorelin, an LHRH agonist that reduces testosterone release from the testis, suppressed nuclear accumulation of mutant AR, leading to rescue of motor dysfunction in male SBMA mice. In a randomized, double-blind, placebo-controlled clinical trial, leuprorelin significantly inhibited accumulation of pathogenic AR protein in the scrotal skin of patients, significantly decreased the level of serum creatine kinase, and suppressed the progression of dysphagia, suggesting that hormonal intervention with LHRH agonist is capable of interfering with the central pathogenesis of SBMA.

Our studies have also indicated several candidates of therapeutics for SBMA. Oral administration of geranylgeranylacetone (GGA) up-regulates the levels of Hsp70 in the central nervous system and inhibits nuclear accumulation

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of the pathogenic AR protein, resulting in amelioration of polyglutamine-dependent neuromuscular phenotypes of SBMA mice. On the other hand, selective inhibition of HSP facilitated degradation of pathogenic AR, leading to improvements of phenotypes in the SBMA mice. Inhibition of Hsp90 is also demonstrated to arrest the neurodegeneration in SBMA mice. Treatment with 17-allylamino geldanamycin (17-AAG), a potent Hsp90 inhibitor, dissociated p23 from the Hsp90-AR complex, and thus facilitated proteasomal degradation of the pathogenic AR in cellular and mouse models of SBMA. 17-AAG thereby inhibits nuclear accumulation of this protein, leading to marked amelioration of motor phenotypes of the SBMA mouse model without detectable toxicity. These HSP-mediated therapies are applicable for other neurodegenerative diseases.

To date, various therapeutic strategies for SBMA have emerged from animal studies, underlining the necessity of clinical studies to verify the results from basic research. Since SBMA is a slowly progressive disorder, appropriate biomarkers would help to improve the power and cost-effectiveness of longitudinal clinical treatment trials.

C17 LOSS OF ENDOGENOUS ANDROGEN RECEPTOR ACCELERATES MOTOR DYSFUNCTION AND ANDROGEN INSENSITIVITY IN A MOUSE MODEL OF SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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X-linked spinal and bulbar muscular atrophy (SBMA) is a slowly progressive motor neuronopathy that also results in mild androgen insensitivity. Evidence from SBMA and other polyglutamine repeat expansion diseases suggests that polyglutamine (PolyQ) expansion causes pathology by imparting a toxic gain of function. However, androgen insensitivity typically involves a deficit of wild-type androgen receptor (AR) function, suggesting that the mild androgen insensitivity of SBMA involves some loss of function as well. In order to investigate the role of normal AR function in SBMA, we compared male mice carrying the human *AR* gene with 100 polyglutamine repeats (AR100) to mice carrying the same transgene, but lacking the endogenous androgen receptor (AR100Tfm). Both AR100 and AR100Tfm mice express the same amount of polyglutamine-expanded AR; however, AR100Tfm mice exhibit earlier onset of weight loss, kyphosis, and hind limb atrophy compared to AR100 mice. AR100Tfm mice also performed worse on the grip-strength test than AR100 mice. In addition to the neuromuscular phenotype, absence of AR uncovered signs of androgen insensitivity. The anogenital distance of male AR100Tfm mice is indistinguishable from a female mouse, whereas the anogenital distance of AR100 mice is normal. AR100Tfm mice also display elevated levels of luteinizing hormone (LH) (3-fold increase, $p > 0.01$); however, testes size in AR100Tfm mice was markedly diminished compared to AR100 animals (28% less, $p < 0.05$). Furthermore, AR100Tfm mice show a decrease in the size of the spinal nucleus of the bulbocavernosis (SNB), which is extremely sensitive to androgen function ($p > 0.05$). We thus hypothesized that the androgen insensitivity results from a diminished ability of polyglutamine-expanded AR to activate transcription. Reporter gene assays using AR-null PC-3 cells reveal a two-fold decrease (relative to wild-type) in the transactivation ability of a polyglutamine expanded AR ($p < 0.05$). These results suggest that the endogenous AR ameliorates pathology in a mouse model of SBMA, and supports a role for the loss of wild-type function of AR in SBMA pathogenesis.

C18 MODULATION OF HSP90 FUNCTION: A MOLECULAR TARGETED THERAPY FOR NEURODEGENERATIVE DISORDERS

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Background: Hsp90 functions in a multi-chaperone complex in folding, activating and assembling its client proteins. Androgen receptor (AR) belongs to the Hsp90 client protein family. Spinal and bulbar muscular atrophy (SBMA) is an inherited motor neuron disease caused by the expansion of the polyglutamine (polyQ) tract within the AR. 17-allylamino-17-demethoxygeldanamycin (17-AAG), a potent Hsp90 inhibitor, is a new derivative of geldanamycin that shares its important biological activities but shows less toxicity.

Objectives: 17-AAG is now in phase II trials as a potential anti-cancer agent because of its ability to selectively degrade several cancer-related client proteins. Additionally, Hsp90 inhibitors also function as molecular chaperone inducers. Several previous studies have suggested that Hsp90 inhibitors could be applied to non-oncological diseases as neuroprotective agents based on their induction of molecular chaperones. Here, we examined the efficacy and safety of 17-AAG in a mouse model of SBMA and its ability to degrade polyQ-expanded mutant AR, since Hsp90 inhibitors have two major activities, preferential client protein degradation and molecular chaperone induction.

Methods: SH-SY5Y cells were plated in six-cm dishes, and each dish was transfected with 8 μg of the vector containing AR24, AR97, or mock (negative control). For cultured cell models, a 1.8 mM stock solution of 17-AAG in DMSO was diluted into fresh medium to give final concentrations of 18–360 nM. For mouse models, 17-AAG treatments were started when mice attained the age of five weeks, and continued until the age of 25 weeks. Male normal littermates, the mice expressing full-length human AR with 24 (AR-24Q mice, 5-5 line) and

97-polyQ tract (AR-97Q mice, 7-8 line) received 50 μl, intraperitoneal injections of 2.5 or 25 mg/kg 17-AAG three times a week on alternate days.

Results: Administration of 17-AAG markedly ameliorated motor impairments in the SBMA transgenic mouse model without detectable toxicity, by reducing amounts of monomeric and nuclear accumulated mutant AR. The mutant AR showed a higher affinity to Hsp90-p23 and preferentially formed an Hsp90 chaperone complex compared with wild-type AR; mutant AR was preferentially degraded in the presence of 17-AAG in both the cell and transgenic mouse models compared with wild-type AR. 17-AAG also mildly induced Hsp70 and Hsp40.

Discussion and conclusions: We reported that administration of 17-AAG significantly ameliorated polyQ-mediated motor neuron degeneration by preferential proteasome degradation of mutant AR. The ability of 17-AAG to preferentially degrade mutant protein would be directly applicable to SBMA and other neurodegenerative diseases. Thus, modulation of Hsp90 function by 17-AAG has emerged as a candidate of molecular targeted therapy for SBMA and probably for other neurodegenerative diseases as well.

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SESSION 3B CARER SUPPORT

C19 IMPACT OF LONG-TERM SURVIVAL ON CARERS: LESSONS FROM DEMENTIA CARE

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The consequences of any devastating progressive condition reach beyond the individual to those who are close to them, yet the well-being of those who become carers is key to the welfare of the 'patient'. Health services therefore need to consider the impact of illness on the whole family and provide support and care for caregivers in their own right. This paper draws on the large body of research on the impact of caregiving for those with dementia, raising parallels and differences from motor neuron disease (MND). The aim is to raise awareness in service providers and stimulate discussion with people with MND and their carers about how best to meet carers' needs.

All of us in close relationships care in a practical and emotional sense for the other. However, in the presence of debilitating illness, the balance changes. The disabled individual is forced into dependence and the able partner into caregiving. The additional activity and responsibility can cause significant stress and distress.

In dementia care, carer stress (subjective burden) is not directly related to the level of practical help (objective burden) but is influenced by other factors. This is also likely to be the case in MND. Pearlin et al.'s stress-process model (1) suggests five principal areas. These are: 1) background contextual factors, such as quality of prior relationship; 2) primary stressors arising directly from care needs (e.g. broken sleep or heavy lifting); 3) secondary role strains arising from clashes with other aspects of life (e.g. competing requirements to work or care for teenage children); 4) secondary intrapsychic strains (e.g. impact on self esteem); and 5) moderators, such as degree of social support. Research has defined particular risks and buffers for dementia carers using this framework and the same would be possible and potentially useful for MND care. The broader conceptual model of Hall (2) which includes consideration of the underlying beliefs and values of the carer may also be of value.

Systematic assessment can lead to helpful psychosocial interventions. A meta-analysis of interventions for dementia carers (3) found benefits for the carer and person cared for. The three main critical success factors were that the intervention should be for both carer and care recipient, should be intensive, and be individually tailored.

This paper will give a tentative outline of stressors that may affect MND carers and suggest directions for future research and intervention.

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C20 THE IMPACT OF AMBIGUOUS LOSS AND EMOTIONAL LABOUR ON FAMILY CARERS

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Background: Assisting family caregivers to deal with the cumulative losses and the emotional cost of caring for a loved one living with ALS/MND is important for the well-being of both the carer and the patient.

Objectives: As part of a larger study, this paper examines family carers' loss and its impact on their ability to provide effective care in ALS/MND.

Methods: Data were collected from family carers through a mail-out caregiver networks scale survey tool, and ethnographic case study of primary caregivers. The tool included items pertaining to relationships with family and friends, financial concerns and recent changes in health. One-way analysis of variance was undertaken across all items of survey data. Family carers participated in audiotaped interviews in their homes and field notes were collected at three time-points over a 10-month period. Description and conceptual ordering were used to analyse the qualitative data.

Results: A total of 74 carers participated in the survey (51 females and 23 males; mean age range 55–64 years). Eighty-six per cent of the carers were spouses, 9.8% were daughters, and 1.4% were sons. When loss and length of time caregiving were analysed, there was a trend towards increasing loss as time spent caring increased, across all survey items. The main predictors of loss were relatives not keeping in touch ($p=0.006$), difficulties in relationships with friends ($p=0.013$) and financial difficulties ($p=0.015$). Fourteen primary carers all of whom were the partner of the patient, volunteered to be interviewed (three males, 11 females; mean age of 59.92 years). Coded interview data correlated with survey findings and provided insight into other losses. Loss of relationships through functional changes and redefinition of roles occurred among family members and their friends. Ambiguity about the changes occurring in their loved one made it difficult for family

members to make meaning of the situation, restricting their ability to manage care. The physical and emotional demands of caregiving left little time for carers to grieve and they tended to keep their feelings to themselves to protect others, thus increasing their emotional labour.

Discussion and conclusions: Many of the losses experienced in ALS/MND are common to other life-limiting illnesses; however, the ambiguous nature of loss arising from the uncertainty associated with disease progression, compounds loss. Family carers' experiences of emotional labour and ambiguous loss often go unnoticed but have a significant impact on their ability to continue to provide care. Health and social care professionals are well placed as people, independent of the family relationships, to support carers in discussing their loss experiences. Strategies such as identifying a possible confidant within the social support network and providing connections to other carers may also enable family carers to manage loss more effectively.

C21 INFORMAL CARERS AND THEIR NEED FOR INCREASED EMOTIONAL AND PRACTICAL SUPPORT

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Background: Literature has shown that informal carers can experience poor mental and physical health while caring for a close relative or friend diagnosed with MND (1). Carers' reporting of ill health often appears to be related to the number of hours spent caring (2) particularly with the introduction of tracheostomy ventilation (3), which can increase both responsibility for, and time spent with, the patient. There has been a recent shift in UK legislation to give greater attention to the needs and wishes of carers (4) and there is now a need to engage with the individuals involved on how services can be improved (5). This raises the question of whether or not professional support should be made more available and in what form.

Objectives: The aim of this study was to examine carers' experiences of looking after someone with MND and how they experienced the support offered to them by friends, family, and the statutory, independent and voluntary services.

Methods: Semi-structured interviews and focus groups were carried out with 23 informal carers of individuals with MND, and transcripts were analysed using a qualitative software package. A disease-specific questionnaire is being developed from the themes identified from these interviews and is being tested by mail throughout its development.

Results: Two of the major themes emerging from the interviews concern the emotional and practical needs of carers of people with MND. Where the carer's informal support circle is insufficient the carer can become isolated resulting in unmet emotional needs and possibly self-reported poor mental health. While health professionals often work outside their remit, there appears to be a general need for early or regularly available emotional support and practical advice. Carers may report better coping skills if relationships with support services were improved, particularly with the general availability of a named coordinator. Training and more practical support may alleviate anxiety, build confidence and improve knowledge of support services.

Discussion and conclusions: Not all carers are proactive or confident enough to actively seek support or advice. Some carers may find it difficult to prioritize their own needs or declare they have problems. They are also diverse in their preferences for support. On the whole, it would appear that standardly available emotional support would be welcome from the professional services, and more practical information and involvement in service provision would aid both carer and patient. Carers' self-reported health may also improve as a result.

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C22 INTENSIVE PSYCHOLOGICAL REHABILITATION IN SMALL FAMILY GROUPS

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Background: ALS patients in Denmark are followed with the help of regular ambulatory control in an interdisciplinary ALS team at hospitals and by staff from the national Rehabilitation Centre for Muscular Dystrophy (RCfM), who consult patients in their homes to clarify the family's need for rehabilitation. The RCfM consultants coordinate the further course of rehabilitation between the ALS team and the local professionals. Within a year of the diagnosis being made, RCfM offers ALS patients and their relatives a weekend course that deals with the medical, mental, and social aspects of the progression of the disease. During the course, patients and their relatives meet each other and exchange experiences in small groups of patients and relatives, respectively. Experience has shown that they

are apprehensive about meeting others with ALS and being confronted with various degrees of the disease, but that they also gain a great deal from the mutual exchange of experience.

About 15–20 patients and their relatives take part in the above-mentioned courses. Patients differ widely in relation to their pathological state, age, and social background, and these circumstances hinder the exchange of experience. We have therefore developed an intensive psychological rehabilitation course for families in small groups that involves ALS patients and their spouses being admitted to hospital for two days, making sure that the participants constitute a homogeneous group with regard to the progress of their disease, age, and social background.

Objectives: to develop a rehabilitation course where:

1. Participants can use each other's experience, reduce the fear of the development of the disease, and consider symptoms and problems that are difficult to handle.
2. ALS patients and their spouses have the opportunity to achieve more mutual empathy and a common understanding of the effect of the disease on family life.

Methods: Four to eight families are referred with the aim of creating a homogeneous group. Participants are interviewed before the course to discover their attitude to the disease and their expectations. An SF36 form is used to describe the quality of their lives. An evaluation interview is held at the end of the course, and after a month there is a telephone interview and another measurement of the quality of their lives with the use of the SF36 form. Consultants from RCfM act as catalysts and supervisors during the course.

Results: The results from the four courses, in which a total of 20–25 families took part, will be presented at the Symposium. The results show that participants felt that the rehabilitation course is what they have got most out of. All participants say that meeting other families in a similar position on such an intensive course has had a great influence on them. They also say that it is important that they took part together with their partners.

Discussion and conclusions: The participants are referred on the basis of a common problem and background. Referral and a clarification of the purpose of the course in relation to the selected group are of decisive importance for the course. Among other things, the results show that an intensive course of psychological rehabilitation in small, homogeneous groups can be a valuable supplement to the other rehabilitation measures.

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SESSION 4A CELL BIOLOGY AND PATHOLOGY

C23 NEUROTOXIC ACTIONS OF SECRETED MUTANT CU/ZN SOD

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Despite a decade of investigation on familial ALS caused by missense mutations in the superoxide dismutase (SOD1) gene, the mechanism of toxicity to motor neurons has remained elusive. The current view is that the toxicity of mutant SOD1 is not related to aberrant copper-mediated catalysis but rather to the propensity of the abnormal protein to aggregate. Surprisingly, recent studies with chimeric mice expressing SOD1 mutants or with mice bearing excisable mutant SOD1 transgenes demonstrated that the toxicity of SOD1 mutants is not strictly autonomous to motor neurons. However, the mechanism by which the toxicity of mutant SOD1 may be transferred from one cell to another has remained unclear.

Although it is well known that SOD1 is a cytosolic protein without specific translocation sequence, a yeast two-hybrid screen led us to discover that chromogranins, components of neurosecretory vesicles, are interacting partners of SOD1 mutants linked to ALS, but not of wild-type SOD1. The existence of such interactions was confirmed by coimmunoprecipitation assays using either lysates from Neuro2a cells cotransfected with chromogranins and SOD1 mutants or from spinal cord of ALS mice. Moreover, confocal and immunoelectron microscopy revealed a partial colocalization of SOD1 mutant with chromogranins in spinal cord of ALS mice. Cell culture studies showed that chromogranins may act as chaperones to promote the secretion of mutant SOD1. Further support for secretion of SOD1 came from fluorescent microscope observation of live cells that revealed considerable distribution of EGFP-fused SOD1, both wild-type and mutant SOD1 in endoplasmic reticulum (ER) labelled by ER-tracker and Golgi markers. Cell-free translocation assay using recombinant SOD1 and microsomes showed that the apo-form SOD1 of both wild and mutant types translocated into microsomes in an ATP-dependent fashion.

We also discovered that extracellular mutant SOD1 can trigger microgliosis and death of motor neurons in culture suggesting a pathogenic mechanism based on toxicity of secreted SOD1 mutant proteins. In this model, it is the burden of extracellular mutant SOD1 in close proximity to motor neurons that would increase the risk of damage. Even though interneurons, motor neurons and astrocytes would be the predominant source of extracellular mutant SOD1 mediated by chromogranin interactions, mutant SOD1 secreted by other pathways in cells such as microglia could also contribute to pathogenesis. Although the deleterious effects of intracellular mutant SOD1 cannot be excluded, our model of toxicity based on secreted mutant SOD1 is compatible with the idea that the disease is not autonomous to motor neurons.

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C24 REDOX SYSTEM UP-REGULATION IN ALS MOTOR NEURONS IS A SURVIVAL MECHANISM UNDER STRESS

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Background: In neurons, peroxiredoxin-II (PrxII) and glutathione peroxidase-I (GPxI) are extremely important enzymes of the redox system that is a crucial antioxidant enzyme defence system and is synchronously linked to other important cell supporting systems.

Objective: To clarify the common self-survival mechanism of the motor neurons in amyotrophic lateral sclerosis (ALS), we investigated PrxII/GPxI-expression dynamics in the motor neurons on the basis of the redox system.

Materials: We have recently produced affinity-purified rabbit polyclonal antibodies against PrxII and GPxI, and have successfully applied it to the paraffin sections. Histological and immunohistochemical studies were carried out on specimens from ALS autopsies and ALS animal models: 40 patients with sporadic ALS (SALS) and seven patients with superoxide dismutase 1 (SOD1)-mutated familial ALS (FALS) from three different families (frameshift 126 mutation, L106V and A4V) as well as four different strains of the SOD1-mutated ALS animal models (H46R/G93A rats and G1H/G1L-G93A mice).

Results: Almost all of the normal neurons in humans and animals expressed the redox system-related enzymes PrxII/GPxI. Although the number of motor neurons in ALS decreased along with disease progression, the number of neurons negative for redox system-related enzymes increased with ALS disease progression. Noticeably, certain residual motor neurons showing redox system up-regulation were commonly found during the clinical course of ALS. In SALS patients, motor neurons showing redox system up-regulation were present three years after

the onset and these up-regulating neurons thereafter decreased in number dramatically, along with the disease progression. In the SOD1-mutated motor neurons in humans and animals, like SALS, certain residual motor neurons without inclusions also showed redox system up-regulation during their clinical course. In addition, some SOD1-mutated motor neurons formed the inclusions where the coaggregation of the redox system-related enzymes with the SOD1 occurred, thereby amplifying the cytoplasmic depletion of these enzymes and resulting in the disruption of the redox system. At the terminal stage of ALS, the breakdown of this redox system up-regulation mechanism in neurons was observed.

Discussion and conclusions: We claim that the residual ALS neurons showing redox system up-regulation would be less susceptible to ALS stress and protect themselves from ALS neuronal death, whereas the breakdown of this redox system would accelerate the process of neuronal death. Our data lead to the development of a new therapy based on redox system up-regulaion for the treatment of ALS, which for over 130 years has had an unknown etiology.

C25 SUPEROXIDE DISMUTASE 1 FROM THE SPINAL CORD OF G93A RATS BINDS TO THE INNER MEMBRANE OF MITOCHONDRIA AND INCREASES MITOCHONDRIAL ROS PRODUCTION

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Mutations in the human Cu/Zn superoxide dismutase (SOD1) gene have been found in 20% of familial amyotrophic lateral sclerosis (FALS) cases. Although the nature of the toxic gain of function in mutant SOD1 has not been identified, it is believed that altered free radical and reactive oxygen species (ROS) generation may be a leading contributing factor to the destruction of motor neurons. Apparent destabilization of the SOD1 molecule, causing enhanced aggregation, is yet another characteristic hallmark of mutant molecule toxicity.

In recent studies mitochondrial localization of mutant SOD1 has been implicated in disease pathology. Although several mechanisms explaining how SOD1 aggregation may cause mitochondrial dysfunction have been proposed, there is a lack of conclusive proof of mutant SOD1 toxicity in mitochondria.

We analysed the molecular features of cytosolic SOD1 extracted from the spinal cord, cortex, cerebellum and liver of G93A SOD1 rats employing modified immunoblotting. This allows us to distinguish the degree of denaturation by binding to a hydrophobic membrane. The results obtained showed clear SOD1 destabilization in the spinal cord. SOD1 stability decreased with disease progression and the amount of destabilized SOD1 peaked at 16 weeks, shortly before the onset of the disease. The binding of destabilized SOD1 to the mitoplasts, isolated from wild-type rat liver, was increased. In parallel, ROS production was significantly elevated in mitoplasts exposed to destabilized SOD1.

The data obtained shed light on the early events in the pathological chain where SOD1, destabilized by mutations, acquires an increased ability to bind to the inner membrane of mitochondria, which in turn directly increases ROS production.

C26 CELL-PERMEABLE PEPTIDE ANTIOXIDANTS AS A NOVEL THERAPEUTIC APPROACH IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Reactive oxygen species (ROS) play a major role in the pathogenesis of neurodegenerative diseases. They are important contributors to necrotic and apoptotic cell death. A major proportion of cellular ROS are generated at the inner mitochondrial membrane by the respiratory chain.

Objectives: In the present study we investigated a novel peptide antioxidant (SS-31) targeted to the inner mitochondrial membrane for its therapeutic effect in neuronal cells stably transfected with either wild-type or mutant SOD1 and in the G93A mouse model of amyotrophic lateral sclerosis (ALS).

Results: SS-31 protected against cell death induced by hydrogen peroxide *in vitro* in neuronal cells stably transfected with either wild-type or mutant SOD1. In G93A ALS transgenic mice, daily intraperitoneal injections of SS-31 started at 30 days of age led to a significant improvement in survival and motor performance. Furthermore, compared to vehicle-treated G93A mice, SS-31-injected mice showed reduced cell loss in the lumbar spinal cord at 110 days of age. We also found a decrease in immunostaining for markers of oxidative stress (4-hydroxynonenal, 3-nitrotyrosine) in the lumbar spinal cord of SS-31 injected animals.

Conclusion: Our data support the assumption that direct targeting of ROS production at the inner mitochondrial membrane and therefore preventing further mitochondrial damage is an interesting new approach to treat neuronal degeneration induced by oxidative stress.

C27 TARGETING OXIDATIVE STRESS AS A POTENTIAL THERAPY FOR ALS

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Background: There is now overwhelming evidence that ALS pathogenesis is, in part, mediated by oxidative damage, although the precise mechanism(s) by which this occurs remains unknown. Given the current lack of effective treatments for ALS, a valid rationale is to investigate the efficacy of anti-oxidants as potential therapies, either individually or as a cocktail.

Objectives: To develop a high-throughput cellular assay of motor neuronal oxidative stress that can be used to screen a drug library for candidate drugs.

Methods: NSC34 mouse motor neuronal cells were transfected with either wild-type or mutant (G37R, H48Q, G93A and I113T) human SOD1 and stable single cell clones were produced. Dichlorofluorescein fluorescence (normalized to cell number to allow comparisons between cell lines) was used to measure reactive oxygen species (ROS) levels in NSC34 cells under basal conditions and during oxidative stress induced by serum withdrawal. Potential anti-oxidant drugs were tested by addition at the time of serum withdrawal. Drug toxicity was tested by measuring ethidium fluorescence from dead cells.

Results: Under basal conditions, NSC34 cells stably expressing four ALS-linked human SOD1 mutations had significantly higher levels of ROS than either untransfected NSC34 cells, or cells stably expressing empty vector or wild-type human SOD1. Untransfected NSC34 cells deprived of serum also exhibited a significant increase in ROS, typically three-fold, providing a simpler assay system. Ebselen, a peroxiredoxin mimic previously shown to increase NSC34 cell survival during serum withdrawal, protected against the observed increase in ROS following serum withdrawal (EC_{50}= 4.8 μM). Using these models, we are screening known anti-oxidant compounds in a targeted approach, and also the Spectrum Collection of 2000 bioactive compounds including known drugs and natural products (MicroSource Discovery Systems) to identify potential therapeutic candidates. Lead drugs identified from this screen will be described.

Discussion and conclusions: The higher oxidative stress levels observed in NSC34 cell lines expressing mutant SOD1 compared to the control cell lines emphasizes the role of oxidative stress in ALS pathogenesis, and provides an *in vitro* assay in which anti-oxidant strategies can be tested. This simple assay can be used to screen large numbers of potential drugs, and the most effective targets will be tested and further developed in more complex models of ALS.

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SESSION 4B IMPACT OF LONG-TERM SURVIVAL

C28 PATHOLOGICAL ASPECTS OF LONG-TERM SURVIVAL IN ALS PATIENTS ON RESPIRATORS

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Amyotrophic lateral sclerosis (ALS) is a progressive, in principle sporadic, neurodegenerative disease of unknown cause affecting adults. Pathologically, loss of lower and upper motor neurons, and degeneration of the corticospinal tract are typical features. Most of the affected patients die within a few years due to respiratory failure.

There has long been a question of whether only the motor neuron system undergoes selective degeneration in ALS. The use of assisted ventilation has meant that respiratory failure is no longer necessarily the end-point of the disease. For example, after the introduction of respiratory support, it has been revealed that in the spinal cord, neurons in Clarke's column and the intermediolateral nucleus are involved primarily in the disease process, and in general tend to disappear with time. The difference in the rate of disappearance between these sensory or autonomic neurons and anterior horn cells represents the rate of degeneration. Therefore, ALS is not simply a motor neuron disease.

What does long-term survival bring to ALS patients on respirators? It has been reported that the entire manifestation of ALS is represented as a totally locked-in state with widespread degeneration, including the motor neuron system. It has also been reported that such lesions are not always a feature in long-surviving ALS patients on respirators. At present, it is certain that at least two pathologic phenotypes (the widespread multisystem form and the non-widespread, rather classical form) are recognizable.

Is artificial respiratory support (ARS) actually useful for ALS patients? In a previous study, we examined 102 autopsy cases of sporadic ALS that occurred between 1962 and 2000 (Brain Research Institute, University of Niigata, Japan). Among the patients, 28 (20 male, 8 female) were given ARS (duration, 5–156 months), and their disease duration (median, 53.5 months) was significantly longer than that (median, 25.0 months) of patients without ARS ($p=0.0002$). With regard to cognitive function, mild dementia was observed in only one patient who received ARS for 114 months; there was no significant correlation between ARS and dementia. In addition, routine pathological examination revealed no apparent expansion of degenerative lesion distribution in the subgroup of cases with ARS. Thus, ARS appears to be a useful choice of conservative treatment when ALS patients wish to live long beyond the point of respiratory failure.

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The Japan ALS Association has contributed greatly to creating a positive approach to living with the disease. However, in Japan, there is now an ongoing debate about whether ALS patients on respirators should be allowed to choose for themselves to continue receiving such support. Pathologic studies suggest that the cognitive function of most ALS patients on respirators remains unaffected for a long period, and that such patients are capable of changing their mind about treatment while receiving ARS.

C29 THE LONG-TERM COMMUNICATION STRATEGY IN PATIENTS WITH TPPV THROUGHOUT THE TOTAL COURSE OF ALS

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This presentation will discuss the long-term communication strategy based on 100 ALS patients with tracheostomy positive pressure ventilation (TPPV) experienced in Tokyo Metropolitan Neurological Hospital.

In Japan, the number of ALS patients with TPPV is more than 30%, and recently the number of patients at home on ventilation has increased. Although respiratory failure in ALS has long been considered the end-point in ALS, the recent clinico-pathological evidence from the patients with TPPV showed that it is not the end-point, but a part of ALS. In general, the respiratory motor group might certainly be the most vulnerable among the five in the voluntary motor system, i.e. extremity, pontine, medullar, respiratory and external ocular. In addition to the voluntary motor system however, the emotional and autonomic systems also might be involved in ALS, and some patients might develop severely impaired communication, i.e. totally locked-in state (TLS) and minimal communication state (MCS). The period of the total course of ALS has changed from onset to respiratory failure, which has been known as Charcot's ALS and we have called 'the previous view of ALS', to a period from onset to involvement of all efferent motor systems, which we have called 'the new view of ALS' and includes the former.

In considering communication strategy throughout the total course of ALS, patients with TPPV should now be included and the physicians should take 'the new view of ALS' in caring for them. Traditional verbal communication may be not effective, and different varied communication needs should be developed during the course of increasing impairments of ALS. Two points of view in the communication strategy should be considered.

The first is physical, extrinsic communication using apparatus, i.e. the development of communication-assistance devices 'just in time' for their essential living such as a switch for nurse-call, etc. The development of these will require good knowledge of the characteristic features and trends of aggravating motor impairments throughout the total course of ALS.

The second is mind-intrinsic communication not using apparatus, i.e. the allocation of decisional authority in the physician-patient-family relationship, and the quality of communication supporting informed decision-making. In Japanese sociocultural tradition, there remains a tendency that the patient's family might defer to and trust in the professional expertise and role of the physician, and the patient is perceived as outside the decision-making process, contrary to the individualistic notion at the heart of autonomy in the West. In Japan, the ample opportunity for physician-patient-family dialogue in the 'fiduciary relationship (Shinnrai-Kankei)' between the physician and the patient's family based on 'the new view of ALS' might have an essential supporting role for patients with TPPV to enhance and foster the enlargement of life, QOL and coping with the inexorable decline in the future.

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SESSION 6A BINDING AND AGGREGATION PROPERTIES OF CU/ZN SOD

C30 IDENTIFICATION OF PROTEINS THAT BIND WILD-TYPE (WT) AND/OR FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (FALS)-LINKED MUTANT CU/ZN SUPEROXIDE DISMUTASE (MTSOD1) USING PHAGE DISPLAY

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Background: There is convincing evidence that FALS-linked MTSOD1 kills motor neurons because of toxicity rather than a deficiency of dismutase activity; however, the basis for this toxicity remains unclear as does effective treatment for this devastating fatal disease. Studies have suggested that MTSOD1 misfolds, and that the mutant protein's aggregation interferes with normal SOD1-protein interactions, sequestering proteins that are important for cell survival.

Objective: To identify peptides that interact with WT and MTSOD1 in order to better understand FALS pathogenesis and to generate reagents that can be used in FALS treatment.

Methods: We used phage display technology to identify peptides that interact with WT and/or MT SOD1. Affinity selection was carried out using phage-displayed combinatorial peptide libraries with purified SOD1 that had been expressed and biotinylated in E. coli as the target. Phage particles displaying peptides were isolated and tested for binding affinity to SOD1 by ELISA. High affinity binding peptides were sequenced, and the predicted amino acid sequence of the peptide ligand was aligned to identify a consensus motif.

Results: We have isolated a number of peptide clones by affinity selection using WT or MTSOD1 as a target. Following sequencing, peptide ligands have been identified that are presumed to be involved in SOD1 binding. Blast search of these peptide ligands identified a number of proteins with a similar consensus sequence. Some of these proteins have been implicated in protein transport and misfolding.

Discussion and conclusions: The pathogenesis of FALS and other neurodegenerative diseases has been hypothesized to involve the formation of aggregates containing the mutant protein relevant to the disease (e.g. SOD1), and the sequestration of proteins, key to the viability of the cell, in these aggregates. For this reason, the identification of SOD1-binding proteins is of importance.

We chose to identify SOD1-binding proteins using phage display technology because of the great power of this method. Our studies identified a number of binding peptides. Sequencing of these peptides demonstrated similar consensus sequences from a number of the peptides. The peptide sequences that we identified are present in proteins involved in protein transport and misfolding. We are presently confirming binding of these proteins to SOD1 by immunoprecipitation. The identification of these proteins may clarify FALS pathogenesis.

We are also testing the ability of the peptides that we have identified to perturb MTSOD1-induced aggregation and/or death in cell culture models. Peptides that interfere with the pathogenicity of MTSOD1 may be useful in FALS therapy.

Acknowledgement: This work was supported by the ALS Association.

C31 INTERMOLECULAR DISULPHIDE BONDS-MEDIATED AGGREGATION OF SOD1 IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Twenty per cent of the familial form of amyotrophic lateral sclerosis (ALS) is caused by mutations in the Cu/Zn superoxide dismutase gene (SOD1) through the gain of a toxic function. The nature of this toxic function of mutant SOD1 has remained largely unknown. SOD1 aggregates are a pathological hallmark in ALS patients with SOD1 mutations and ALS mouse models that overexpress ALS-associated SOD1 mutants. The relevance of these SOD1 aggregates to development of ALS and the fundamental molecular mechanism by which the SOD1 mutants form aggregates are not clear.

Objectives: The objectives of this study were to elucidate 1) the possible causal relationship between SOD1 aggregates and development of ALS; and 2) the molecular mechanism of how the SOD1 forms aggregates.

Methods: Single and double transgenic mice overexpressing wild-type and various SOD1 mutants were developed. SOD1 aggregates were analysed using biochemical, immunological and pathological methods.

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Results: We found that wild-type SOD1 exacerbates the ALS phenotype in double transgenic mouse models overexpressing $SOD1^{G93A}$ and $SOD1^{L126Z}$. This phenomenon is similar to that previously reported in prion disease. We also found that w-t SOD1 can convert an unaffected phenotype to an ALS phenotype in mutant SOD1 transgenic mouse model overexpressing $SOD1^{A4V}$ in a dose-dependent manner. Further analyses of the single and double transgenic mice revealed that conversion of mutant SOD1 from soluble form to an aggregated and detergent-insoluble form was associated with the development of an ALS phenotype in transgenic mice. Conversion of w-t SOD1 from soluble form to an aggregated form correlates with exacerbation of the disease or conversion to a disease phenotype in the double transgenic mice. We found that this conversion, observed in the mitochondria of the spinal cord, involved formation of insoluble SOD1 dimers and multimers that are cross-linked through intermolecular disulphide bonds via oxidation of cysteine residues in SOD1.

Discussion and conclusions: The aggregated and detergent-resistant form of SOD1, either mutant or wild-type, is associated with ALS and is apparently the pathogenic form of the protein. These aggregates are predominantly observed in mitochondria of the spinal cord of the ALS mouse models and are formed by intermolecular linked SOD1 dimers and multimers via oxidation of the cysteine residues in SOD1. Thus, our findings provide evidence of direct links between oxidation, protein aggregation, mitochondrial damage and SOD1-mediated ALS with possible applications to the ageing process and other late-onset neurodegenerative disorders, such as prion disease and Alzheimer's disease.

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C32 STRUCTURE BASED DESIGN OF SOD-1 AGGREGATION INHIBITORS: IMPLICATIONS FOR DEVELOPMENT OF A NEW CLASS OF ALS THERAPEUTICS

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Background: Aggregation of mutant forms of the housekeeping gene SOD-1 has been implicated in motor neuron cell death in a subset of the familial form of ALS. The downstream mechanisms following aggregation that lead to death of motor neurons are poorly understood and have proven to be difficult targets for drug discovery. We propose a novel approach for development of ALS therapeutics based on the concept of 'native state protein stabilization'.

Objective: In the absence of any consensus for the mechanism of SOD-1 mediated toxicity, stabilization of the native state of the protein using small molecules is a viable strategy for developing ALS therapeutics. We propose to design molecules that would be structure dependent rather than mechanism dependent. Stabilizing the SOD-1 dimer would automatically eliminate all downstream events that eventually lead to neurotoxicity.

Methods: Based on this concept, we initiated an *in silico* screening programme to find drug-like molecules that would stabilize the SOD-1 dimer. About 1.5 million drug-like molecules from commercial databases were screened against a dimer interface cavity. 100 molecules with the highest predicted binding affinity were tested experimentally *in vitro* and 15 out of the 100 molecules significantly inhibited aggregation and denaturation of a number of common FALS mutants such as A4V, G85R, S134N, G93A and H46R. All of the mutants exhibited near wild-type-like stability in the presence of these 15 molecules.

Results: Close examination of these 15 hits led to the identification of an aza-uracil pharmacophore around which a structure-activity relationship (SAR) was designed using a set of 50 related compounds with drug-like features (Lipinski based). The compounds were tested both *in vitro* (biochemical assays) and in mouse neuroblastoma (N2A) mutant SOD-1 cell lines. A second assay was designed to improve binding-selectivity for these compounds in serum and CSF to generate highly target-specific molecules (designer drugs). A set of compounds with drug-like properties were obtained from the secondary screens. The molecules were also found to be fairly non-toxic in cell culture and preliminary mice studies. Currently, some of these molecules are being tested in a FALS transgenic mouse model.

Conclusions: We have obtained set of molecules capable of blocking SOD-1 aggregation, which also have drug-like properties and have low levels of toxicity in cells. While there are no viable ALS drugs and not much progress is expected from the private sector since the market is too small to justify an intensive effort, we propose a model where at least part of the drug development procedure can be undertaken in an academic environment. We hope such an effort will promote collaboration with industry towards a much-needed cure.

C33 ULTRA-HIGH AND ATOMIC RESOLUTION STRUCTURES OF CU/ZN SOD1 AND ITS FALS MUTANTS

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About 10% of all ALS cases are familial (FALS). Dominant inheritance of point mutations in Cu/Zn superoxide dismutase (SOD1) are involved in about 20% of FALS. This well characterized subset of FALS offers the best chance of understanding the origin of the disease and arriving at a general strategy for a cure. SOD1 is a crucial component of the cellular response to oxidative stress, catalysing the dismutation of the superoxide radical to hydrogen peroxide and water. Recently, the toxic gain-of-function properties of SOD1 including misfolding, unfolding and/or aggregation have been implicated as a probable cause for at least a fraction of the sporadic cases of ALS. Hence, it is essential to not only gain detailed insight into the molecular behaviour of the FALS mutant molecules but also the wild type under different conditions.

Knowledge of protein structures and how they are affected by, for example mutations and/or loss of metals, is a prerequisite for developing suitable compounds and drugs which may inhibit the disease-causing character. X-ray crystallography provides the most detailed picture of the atomic arrangement within a protein molecule. Through ours and others' efforts, there is clear evidence that 1) mutations impair the global and/or local stability of SOD1 dimer molecules; and 2) that metal loss in SOD1 results in protein aggregation. We are looking to identify potential 'drug binding pockets' using the catalogue of structures we have determined; we are also searching for compounds with corrective properties. To this end we have continued to improve the resolution of the structures of the wild-type and FALS mutant molecules. Thus, for example, we have obtained the structures of recombinant wild-type SOD1 to 0.8 Å[1] (known as ultra-high resolution where even hydrogen atoms can be seen) and A4V to 1.15 Å (known as atomic resolution), to examine the structures of the wild-type enzyme and its A4V mutant. At such resolutions, it is possible to observe protein conformational substates that are occupied for only a small fraction of time. It is also possible to distinguish between regions in the molecule that adopt conformations along a continuous range, or that jump between two or more distinct conformations. Such information would contribute towards identifying 'binding pockets' and aid the search for compounds with corrective properties. These and early results for ligand/compound binding will be presented.

[1]There are currently 36,531 structures of protein molecules and their derivatives in the protein data bank. Of these only 16 structures are known to a resolution at or better than this resolution.

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SESSION 6B NUTRITIONAL ASSESSMENT AND SUPPORT

C34 MODELLING ENERGY EXPENDITURE IN ALS: A PROSPECTIVE LONGITUDINAL STUDY

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Background: Maintaining optimal nutrition for an ALS patient requires knowledge of food intake and energy (calorie) expenditure at various stages of the illness. Weight loss and determination of BMI are indirect indices of malnutrition since loss of body mass may be due to muscle atrophy and not undernutrition. The currently used energy predictive equations, such as the Harris-Benedict equation, are not disease- and state-specific for ALS. Without a specific equation for ALS, one might inadvertently recommend under- or over-feeding.

Objectives: The goal of our study was to prospectively develop empiric equations to accurately predict energy expenditure in ALS patients in various functional states as their disease progresses.

Methods: We use the doubly-labelled water (DLW) method to determine the absolute caloric expenditure over a 10-day period (Total Daily Energy Expenditure, TDEE), which serves as the reference against which we will model various clinical factors to predict TDEE. To begin the modelling process, we determine lean body mass (LBM) using DXA and BIS methods to estimate basal metabolic rate (BMR) using the Wang and other equations. We determine: resting metabolic rate (RMR) using indirect calorimetry in sitting and supine positions, physical activity using accelerometers and the Bouchard rating of physical activity by the subject, and clinical data using rating scales of cramps, spasticity, fasciculations, and pseudobulbar behaviours which represent potential sources of caloric expenditure that do not result in functional movement.

Results: To date, we have enrolled 39 of a projected 100 subjects at five medical centers. The mean age was 58.6 years; 57% were male, 87% were Caucasian, 27% had unimpaired ambulation, and 37% had an entry FVC $>80\%$ of predicted, indicating that we successfully recruited a broad clinical spectrum of ALS subjects. Determination of LBM by DXA and BIS was highly correlated. The difference between TDEE and measured RMR varied widely between 181 and 1461 kcal/day (774 ± 392 kcal/day). The difference between TDEE and the predicted RMR using the Harris-Benedict equation varied between -67 and +1817 kcal/day (665 ± 517 kcal/day). Other predictive equations yielded similar discrepancies.

Conclusions: The differences between measured or predicted RMR and TDEE represent the calories expended on a daily basis by the ALS patient that are not accounted for using these standard methods in clinical nutritional practice. As more patients are studied and longitudinal measurements obtained over 12 months, we will be able to analyse the contribution of the various clinical factors to account for the discrepancies between the absolute TDEE and the predicted energy needs using the standard approaches. We plan to develop new predictive equations based on these data and also disease-specific modifications of the widely-used Harris Benedict equation. The results of our study will standardize nutritional care in clinical practice and in drug trials.

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C35 VALIDATION OF BIOELECTRICAL IMPEDANCE SPECTROSCOPY FOR MEASURING LEAN BODY MASS AND TOTAL BODY WATER IN ALS

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Background: Malnutrition due to energy (calorie) imbalance, and dehydration, are common in amyotrophic lateral sclerosis (ALS). Cachexia due to loss of lean body mass (LBM) and fat mass is an ultimate consequence of the disease. Bioelectrical impedance spectroscopy (BIS) is a non-invasive and relatively inexpensive technique for measuring body composition and total body water (TBW), variables that are essential for the understanding of body composition and caloric balance.

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Objective: To prospectively validate the accuracy of BIS in measuring LBM and TBW, against the reference gold standard techniques of doubly labelled water (DLW) and dual X-ray energy absorptiometry (DXA), in ALS.

Methods: We are currently undertaking a longitudinal, prospective study of caloric balance and changes in body composition in patients at five ALS centers. For the purposes of this validation study, data on demographics, disease characteristics, body composition measured by BIS, DXA and DLW; and TBW estimated by BIS and DLW are being collected.

Paired *t*-tests and Pearson's correlation coefficients were used to compare LBM measured by DXA and BIS, and TBW measured by DLW and BIS. The absolute and percentage mean difference from data obtained by the reference techniques was recorded.

Results: To date, we have data available on 26 of an expected 100 patients. There were 54% males and onset was bulbar in 35% of patients. Other mean $\pm$ SD values were: age 57 ± 10 years, disease duration 26 ± 19 months, BMI $25 \pm 5 kg/m^2$, ALSFRS 35 ± 6 and FVC $74 \pm 15\%$ of predicted.

The mean $\pm$ SD LBM-BIS was 2.4 ± 3.4 kg lower than LBM-DLW (46.0 ± 14.0 kg vs. 48.4 ± 12.9 kg, mean difference -5.6%, $p=0.002$) ($n=25$); and 1.9 ± 4.0 kg lower than LBM-DXA (46.8 ± 14 kg vs. 48.7 ± 13.7 kg, mean difference -4.1%, $p=0.04$) ($n=22$). Pearson's correlation between LBM-BIS and LBM-DLW was $r=0.97$ ($p<0.001$), and between LBM-BIS and LBM-DXA was $r=0.96$ ($p<0.001$).

The mean $\pm$ SD TBW-BIS was 0.2 ± 4.1 l higher than TBW-DLW (35.7 ± 11.0 l vs. 35.5 ± 9.3 l, mean difference 0.3%, $p=0.84$) ($n=26$). Pearson's correlation between TBW-BIS and TBW-DLW was $r=0.93$ ($p<0.001$).

Discussion and conclusion: Compared to DLW and DXA, BIS underestimates LBM by about 4–5% in ALS; nevertheless, LBM estimated by BIS is highly correlated with that measured by the gold standard techniques of DLW and DXA. BIS is as accurate as DLW in estimating TBW. Thus, BIS can be reliably used in studies of caloric balance in ALS.

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C36 COMPLIANCE AS IT RELATES TO SPECIFIC ELEMENTS IN THE PRACTICE PARAMETERS FOR PEG PLACEMENT

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Background: According to the practice parameters of the American Academy of Neurology, percutaneous endoscopy tubes (PEG) should be recommended for accelerated weight loss and symptomatic dysphagia soon after the onset of these difficulties. However, terms such as 'accelerated', 'symptomatic', and 'soon' leave room for interpretation. It is not known how varying degrees and types of clinical findings affect compliance.

Objective: To determine how specific clinical parameters affect compliance for PEG intervention.

Methods: We reviewed records from 63 patients who died after being cared for at a multidisciplinary ALS center. PEG had been recommended for 52 patients (81%) before death. These patients were placed in three groups: 1) compliers (C), $n=24$, PEG within two months of recommendation; 2) eventual compliers (EC), $n=18$, initially refused PEG, placed more than two months later; and 3) non-compliers (NC), $n=9$, recommended but not received before death. Data from all visits, from the initial recommendation to death, were reviewed (dates of recommendation, placement, and death; gender; weight loss; per cent ideal body weight and usual body weight; FVC; ALSFRS scores for swallowing and saliva). Features were compared at the time PEG was offered; C and EC were also compared at the time PEG was eventually placed. A logistic regression model was used to determine which combinations of independent variables effectively predict PEG compliance. Odds ratios were calculated for each independent variable to determine how well they predict the occurrence of PEG placement.

Results: Significant differences at the time PEG was offered were found in ALSFRS bulbar subscores between EC and C (mean scores for swallowing EC=2.8, C=2.2, $p=0.02$ and for salivation EC=2.9, C=2.2, $p=0.02$). There were no differences in other variables. At the time the PEGs were placed, there were no statistical differences in any parameter between C and EC. The initial EC group took a mean of 252 (60–852) days to comply with the initial recommendation and lost an additional 4% (mean) of body weight (range 18% to gained 11%) during the interval. There were no significant differences between EC and NC at the time of recommendation for PEG, although NC had higher mean ALSFRS scores for swallowing (3.3) and saliva (3.4). At the time of death, the mean swallowing score in NC was 2.8.

Discussions and conclusions: Compliance with recommendations for PEG appears to depend on the degree of dysphagia. Patients who fulfil practice parameters for PEG may be reluctant to comply if they perceive swallowing is relatively intact, regardless of weight loss or respiratory difficulty. It is not yet clear if non-compliance is related to actual dysphagia or under-reporting of severity.

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SESSION 7A INTERPRETATION OF DATA FROM SOD1 MODELS

C37 THE SOD1 MOUSE AS A MODEL OF SPORADIC ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive disorder of the motor system leading to death within two to five years. Although most cases of ALS are sporadic in origin, 10–15% are inherited. Among the population with familial ALS, 15–20% possess a mutation in the SOD1 gene that codes for the enzyme Cu/Zn superoxide dismutase. The phenotypic variability observed between affected FALS individuals from the same family, in particular in onset and severity, lends strong support to the likelihood that there are important additional genetic modifiers of the disease.

Transgenic (Tg+) mice expressing a mutated human SOD1 gene (G93A) demonstrate clinical symptoms and neuropathological findings similar to human ALS. We have observed that the genetic background of these hSOD1(G93A) Tg+ mice can affect disease phenotype (onset and survival). Similar observations in related mouse models of motor neuron degeneration have been reported by others. Hence, we proposed that these differences could be used to identify genes that modify severity in this species and that potentially contribute to susceptibility or neuroprotection in human motor neuron degenerative disease.

Our collaborative work is based on these hypotheses: 1) There are variants of genes between mouse strains that alter phenotype; 2) These genes are likely to alter phenotype by influences on one of several pathways important to the mechanism of neuronal death in the G93A SOD Tg+ mouse including changes related to intracellular processing of mutated SOD1, altered inflammatory responses, altered oxidative stress, altered glutamate clearance, or altered cell death.

Thus far, we have bred the Tg on five genetic backgrounds, C57Bl/6J (abbreviated B6), SJL, C3H, B10, and DBA. Compared to the mixed background (B6 x SJL)Tg+, mean survival on the SJL and C3H backgrounds are significantly shorter while the survival on the B6 background is longer. In addition, gender-based influences on phenotype include the fact that Tg+ females live longer in some congenic lines (SJL, C3H) but not others (B6); thus the congenic lines may also provide insight into gender-based differences in human disease. The B6 and SJL congenic lines were used to create an F2 intercross, and a genome scan revealed significant linkage with lifespan to chromosome 2, 5, 10, and 15, and to disease latency on chromosome 2.

Because the human FALS and SALS clinical phenotypes are indistinguishable, the two phenotypes are likely to share important disease mechanisms; thus, identification of the gene(s) and related pathways based on these studies with the G93A Tg+ mice should lead to an increased understanding of the disease mechanisms and design of new treatment strategies pertinent to all ALS/MND patients.

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C38 DESIGN, POWER AND INTERPRETATION OF STUDIES IN THE STANDARD MURINE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Identification of SOD1 as the mutated protein in a significant subset of familial amyotrophic lateral sclerosis (FALS) cases has led to the generation of transgenic rodent models of autosomal dominant SOD1 FALS. Mice carrying 23 copies of the human $SOD1^{G93A}$ $SOD1^{G93A}$ transgene are considered the standard model for FALS and ALS therapeutic studies. To date, there have been at least 50 publications describing therapeutic agents that extend the lifespan of this mouse. However, no therapeutic agent has shown corresponding, substantial, clinical efficacy. We used computer modelling and statistical analysis of 5429 SOD1G93A mice from our efficacy studies to identify several critical confounding biological variables that must be appreciated and should be controlled for when designing and interpreting efficacy studies. Having identified these biological variables we subsequently instituted parameters for optimal study design in the SOD1G93A mouse model. We repeated several of the major animal studies (minocycline, creatine, ritonavir, celecoxib, sodium phenyl butyrate, ceftriaxone, WHI-P131, thalidomide, and riluzole) using an optimal study design and found no survival benefit in the SOD1G93A mouse for any compounds administered by their previously reported routes and doses. The presence of these uncontrolled confounding variables in the screening system, and the failure of these drugs to demonstrate efficacy in adequately designed and powered repeat studies, leads us to conclude that the majority of published effects are most likely measurements of noise in the distribution of survival means as opposed to actual drug effect.

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C39 AN ARRAY-BASED APPROACH TO IDENTIFY BIOMARKERS FOR ALS

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Background: The SOD1 G93A transgenic mouse model is the standard tool for the investigation of new therapeutic candidates for ALS. These transgenic mice are asymptomatic at 60 days of age, and display progressively worsening symptoms from approximately 90 days until death, which occurs on average at 136 ± 10 days. Our studies suggest that a minimum of 24 animals are required per group because of variability in the survival of the animal. As such, it would be highly desirable to have a surrogate endpoint that could facilitate drug screening using fewer animals per group.

Objective: The primary goal of this study is to establish a panel of biomarkers for disease progression in the SOD1 G93A mouse. In achieving this goal we will further our understanding of the molecular mechanisms in ALS and also generate a diagnostic tool for evaluating therapeutic candidates. In addition, if a disease progression-modifying compound also induces altered expression levels of a particular gene, then a target validation approach will be initiated to determine if that gene and/or gene product is a therapeutic target.

Method: We analysed spinal cord tissue from SOD1 G93A mice at various time points during the mouse lifespan. We used a real time-quantitative PCR based, targeted array approach to systematically examine expression levels of functionally categorized genes. More focused RT-qPCR assays were performed to confirm expression changes observed by the array approach.

Results: Over the time course examined, we detected increasing RNA levels of: the glial marker Gfap (4.1-fold), proteasome subunits Psmb8 and Psmb9 (5.8- and 3.8-fold, respectively), apoptosis associated cyclinD1 (3.7-fold), and inflammatory mediators Tnf alpha (9.5-fold), Trem2 (11.6-fold) and Tyrobp (12.6-fold), among others. Decreasing RNA levels were measured for, among others: the neuronal markers NSE/Eno2 and NF-M/Nef3 (2.0- and 3.1-fold, respectively), the molecular motor subunits Kif3 alpha and Dnchc1 (1.7-fold and 2.3-fold, respectively). The genes, whose RNA reproducibly changed over the SOD1 G93A mouse lifespan, were categorized as progressively changing (over the course of disease) or late stage (changed at or near end stage of disease). To assemble the biomarker panel we included or excluded genes based on their temporal expression. To follow up results that suggest a gene's product is a target for therapeutic intervention, immunoblotting, ELISA and in situ hybridization experiments were performed to examine protein levels of genes of interest.

Conclusion: The end product of this effort is a panel of genes which may be used as a diagnostic tool to monitor disease progression in the SOD1 G93A mouse and to examine the effects of therapeutic candidates on that progression. By-products of this work are potential targets for therapeutic intervention in ALS.

C40 GUIDELINES FOR THE PRECLINICAL EVALUATION OF PHARMACOLOGICALLY ACTIVE DRUGS FOR ALS/MND: REPORT ON THE 142nd ENMC INTERNATIONAL WORKSHOP

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A transgenic animal model for anterior horn cell loss was established in 1994. This model is based on the insertion of a high copy number of disease-causing human Cu/Zn SOD mutations into the intact mouse genome. It serves to establish hypotheses for the pathogenesis of anterior horn cell death, but also to test potential pharmacological approaches to therapy in human ALS. To date, more than 100 (published and unpublished) compounds have been tested in this animal model, with a large proportion of them being successful. However, it has proved to be difficult to translate these therapeutic successes in the animal model into human trials. In addition, a number of disease-modifying strategies have been difficult to reproduce, even by the same group. On the other hand, the step from mice to men means a huge investment for the sponsors of clinical trials and the scientific community. Therefore, establishment of standard methods for drug testing in ALS models is mandatory. In this workshop, clinical and preclinical researchers established in the field of ALS/MND met in Holland in March 2006 in order to establish guidelines for the community for drug testing in mouse models.

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SESSION 7B PALLIATIVE AND END OF LIFE CARE

C41 PALLIATIVE CARE BEYOND CANCER

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From its inception in the 1960s, palliative care has focused primarily on the care of terminally ill cancer patients, with these patients still making up 92% of those receiving care from hospice and specialist palliative care services in the UK. There has, however, always been recognition that the principles and practice of palliative care might also benefit other patients with life-threatening long-term conditions and some, particularly in the UK those with ALS, have received this care.

There is now increasing acknowledgement in many countries that palliative care should be provided on the basis of need, not diagnosis, challenging the cancer focus. There is also growing evidence of the presence of palliative care needs (including pain and other symptoms, psychological and spiritual distress, and family distress) beyond cancer; these needs are particularly apparent in people living with ALS.

Addressing these needs presents significant challenges, however. These include the reluctance of some palliative care providers to become involved with patient care beyond cancer, and reluctance of some of those currently caring for these patients to consider that palliative care should be involved, at least until the very end of life. These attitudes adversely affect the partnership working between palliative care and other health and social care agencies (for example, between neurological, rehabilitative and palliative care services) which is essential to palliative care beyond cancer, and which is in itself challenging to achieve. Other challenges include funding issues and public perceptions of palliative care.

Models of collaborative working which address some of these challenges will be presented, and the importance of services working together to meet patient and family need will be emphasized. By moving beyond cancer, palliative care specialists can bring their particular expertise together with that of those specialists currently caring for patients with long-term life-threatening conditions to further improve the quality of the time remaining to these patients.

C42 FACTORS DETERMINING EUTHANASIA AND PHYSICIAN-ASSISTED SUICIDE AMONG PATIENTS WITH ALS IN THE NETHERLANDS (2000–2005)

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Background: Previously, we have reported that during the period 1994–1998 one in five patients with ALS in the Netherlands died as a result of euthanasia or physician-assisted suicide. Euthanasia and physician-assisted suicide were not associated with any of the disease or care-related characteristics or with income or educational level.

Objectives: 1) to determine whether rates of physician-assisted death are increasing (slippery slope); 2) to determine in more detail whether relationships exist between quality of care, quality of life, symptoms and physician-assisted death according to spouses of deceased patients; 3) to determine the reasons for physician-assisted death according to spouses.

Methods: We identified physicians of 308 patients who were diagnosed as having ALS and died between 2000 and 2005 in the Netherlands. The physicians were asked to complete a validated questionnaire to investigate the medical end-of-life decisions that were made and possible care-related, clinical and social correlates. Of 282 eligible physicians, 215 returned the questionnaire (76%). Spouses of patients were contacted also, and 75% returned the questionnaire.

Results: Euthanasia occurred in 36 of 215 patients (17%) and physician-assisted suicide in three of 215 patients (1%). For comparison, in 1994–1998 we reported these frequencies as 17% and 3%, respectively. Euthanasia and physician-assisted suicide were not associated with any of the disease or care-related characteristics or with income or educational level. In addition, according to the information provided by the spouses, there were no relationships with the patient feeling a burden, availability of aids and appliances, or depressive symptoms. The main reasons for physician-assisted death were fatigue, loss of dignity, and dependency. These reasons were more frequent in the physician-assisted death group as compared to the patients who died after another medical end-of-life decision (e.g. to forego or withdraw treatments, or to provide opiates to relieve symptoms with a potential life-shortening effect). Reasons that were not increased

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were: feeling a burden, depression, fear of choking, and pain.

Conclusions: We did not find signs of a slippery slope with regard to physician-assisted death among patients with ALS in the Netherlands. Furthermore, analysis of quality of care, quality of life and symptoms, including depression, did not show an association. Main reasons for physician-assisted death were fatigue, loss of dignity, and dependency.

More results will be presented and discussed, also in relation to standards of palliative care in the Netherlands.

C43 FOLLOW-UP SURVEY OF PATIENTS WITH ALS/MND INTERVIEWED ABOUT SELF-DETERMINATION

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Background: At the 16th International ALS/MND Symposium in Dublin, we reported the results from semi-structured interviews revealing that many ALS/MND patients in our prefecture of Japan seemed to think about self-determination to choose the options for the medical management at the advanced stages of their disease. Since then, we have followed most of the cases directly at our clinic, or indirectly via the Liaison Council of the Networking for the Patients with Intractable Neurological Diseases in our prefecture.

Objectives: To study the present stage of the illness of the patients and the status of the self-determinations after the interviews.

Methods: Twelve out of the 14 ALS/MND patients who had received semi-structured interviews from November 2004 to March 2005 were submitted for our survey. The patients, families or health care professionals were interviewed again between February 2006 and April 2006.

Results: Nine patients were still alive and three were deceased after the previous interviews. Four patients with a mechanical ventilator with tracheostomy (TMV) were still alive, although their conditions had gradually deteriorated. The ALSFRS-R scores of the subjects decreased from 19.1 ± 16.5 to 15.9 ± 16.1 during this interval (average $\pm$ standard deviation). In total, six patients made their own advance directives. Two of them revoked the directives and chose TMV. All three of the deceased patients did not want to receive it and died at home. Two of the deceased had written advance directives that were not used, as an ambulance was not called. One patient recently made a written directive to resolve an intrafamilial conflict.

Discussion and conclusions: It may be exceptional that half of the patients made advance directives in our small series and none of them were involved in a legal case to date. Patients who thought about self-determination seem to have chosen written documents rather than verbal expression only for resolving their internal conflicts, not for using it as a legal document.

Some guidelines or legislation for the scheme to support the patients' wishes seem to be necessary in Japan before serious legal problems occur for patients, families or health care professionals.

C44 PEOPLE LIVING WITH ALS/MND TELL THE DIAGNOSIS STORY: WHAT HAPPENED BEFORE THEY KNEW

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Background: Being told that one has a life threatening disease is shattering, but for some people it comes as a relief, following as it does the years of uncertainty and traumatic experiences that lead to diagnosis. The need to debrief the experience is paramount before the story of living with the disease can be told.

Objectives: The purpose of this paper is to describe the extended and often demoralising process of diagnosis for people with ALS/MND.

Methods: Grounded theory methodology was used to explore the life and world of people diagnosed and living with ALS/MND. Data were collected via in-depth interviews with 25 people with the disease, their stories and photographs, poems and books they identified as important, and field notes. The textual data were analysed using constant comparative analysis. All people who volunteered were included in the study. Many participants with communication challenges worked with the researcher to tell their stories.

Results: Participants recounted the processes they experienced prior to the time when they were finally given a reason for the perplexing behaviour of their bodies. The diagnosis story was revealed as a sequence of: 'recognizing a problem', 'seeking medical help', 'being on the diagnostic roundabout', 'confirming ALS/MND', 're-evaluating life and the future', then 'living with ALS/MND'. Consequences included a loss of trust in the competence of the health care system, which had implications for seeking help later when living with the disease.

Discussion: Participant distress seemed to have more in common with the stress linked to post-traumatic stress disorder (PTSD). Participants continued to relive the diagnosis experience in their dreams and daily lives many months after diagnosis, which impacted negatively on their well-being. For this group of people, the diagnostic process itself was the traumatic stressor. It seemed that telling their stories gave them the opportunity to debrief and have their words recorded. Debrief support is recommended when

the ALS/MND diagnosis is finalized, and continued, to prevent long-term reliving of the diagnostic process.

Conclusion: Health professionals continue to address the issues around the process of giving the 'bad news' of ALS/MND. This 'diagnosis story' may provide additional guidance in addressing the process so as to limit potential harm and promote well-being for people with the disease and their families.

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C45 PALLIATIVE CARE: A CHANGING ROLE IN MND/ALS

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Background: Although specialist palliative care has been involved in the care of people with MND/ALS, the roles are changing. Due to increasing pressures on services there is a tendency for them to be involved later in the disease progression. There is also the possibility of developing relationships and collaboration with neurological services in the care of all people with advancing and progressive neurological disease.

Objectives: The aim of this paper is to look at the development of a collaborative approach in the care of people with MND/ALS.

Methods: A literature review and discussion will be presented showing the possible move towards a collaborative approach in care

Results: As there is increasing pressure within the UK to be involved with the palliative care of more patient groups there is the need to re-evaluate the role of specialist palliative care. There have been other studies showing the palliative care needs for multiple sclerosis and Parkinson's disease, revealing that there are both symptom and psychosocial issues that need to be addressed.

MND/ALS is different, in that the rate of deterioration is greater. However this is changing with the increased use of ventilatory support and gastrostomy, and life expectancy may be increased. Moreover, the expectations of the patient and family may become over-optimistic, with reduced awareness of the possibility of deterioration and death.

There may be an increasing role for palliative care services to be involved alongside other neurological or rehabilitative services and to take a greater role as the end of life is approached. This would allow involvement and support in decision making by patients and families.

Discussion: The paper will look at these changing roles and the need to re-evaluate the collaborative approach in the care of people with MND/ALS. This may involve increased collaboration at times, when decisions are made, and reduced contact when the patient is relatively more stable. There will be the need to ensure that, despite all the interventions that are made, the patient and family are still aware of the potential for deterioration and the need to anticipate and prepare for the deterioration at the end of life.

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SESSION 7C SURROGATE MARKERS AND OUTCOME MEASURES

C46 TEST-RETEST AND INTER-EXAMINER RELIABILITY OF AUTOMATED MOTOR UNIT NUMBER ESTIMATION (MUNE): COMPARISON OF MULTIPLE POINT STIMULATION AND STATISTICAL METHOD

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Background: Motor unit number estimation (MUNE) is a useful tool to assess the disease progression of ALS and other motor neuron diseases; however, it has not become popular in Japan because of its complicated procedures. Recently, two automated systems of common techniques for MUNE became available for clinical use – the multiple point stimulation (MPS) and the statistical method. This study compares these two automated techniques for MUNE to determine which is more reproducible.

Objectives: Fourteen normal controls aged 23–45 years were measured. Two neurologists skilled in electromyography participated in this study as the examiners.

Methods: Surface recorded motor unit action potentials (SMUPs) of the right hypothenar muscle (ADM) were measured on normal controls. For multiple point stimulation, 10 different threshold SMUPs were recorded and averaged automatically with the MPS MUNE program on the NIHON KOHDEN MEB-9104. For the statistical method, mean SMUP amplitude was measured at several stimulus levels, typically spanning $>40\%$ of CMAP amplitude range, with the statistical MUNE program on the Nicolet Viking IV. Both techniques were performed by two examiners twice, and results averaged, electrodes changed, and all recording repeated, totalling eight times for each muscle.

Results: By the statistical method, the estimated motor unit number for normal controls was 135 ± 19 (mean +/− standard deviation) for examiner 1 and 131 ± 25 for examiner 2. There was no statistical significance in these numbers, and inter-examiner correlation coefficient was 0.857 ($p < 0.05$) for statistical method. Test-retest correlation coefficient was 0.772 ($p < 0.05$) for examiner 1 and 0.857 ($p < 0.05$) for examiner 2.

By the MPS method, the estimated motor unit number for normal controls was 194 ± 41 for examiner 1 and 183 ± 53 for examiner 2. There was no statistical significance in these numbers, and the inter-examiner correlation coefficient was 0.687 ($p < 0.05$) for the MPS method. Test-retest correlation coefficient was 0.736 ($p < 0.05$) for examiner 1 and 0.739 ($p < 0.05$) for examiner 2. Inter-method correlation coefficient was 0.567 ($p < 0.05$) for examiner 1 and 0.389 (ns) for examiner 2.

Discussion: The statistical method showed significantly smaller number than the MPS method by both of the examiners, and the inter-method correlation coefficient was not significant. While the MPS method depends on discrimination of each SMUP, the procedure is not fully automated and needs more sophisticated skills.

Conclusions: Both the statistical method and the automated MPS method were reproducible, and easily used in clinical practice. The estimated motor unit numbers are not always compatible between the two methods, and mixed use is not recommended.

C47 THERE IS EVIDENCE FOR WHOLE BRAIN ATROPHY IN PRIMARY LATERAL SCLEROSIS AND IT APPEARS TO BE RELATED TO GREY MATTER ATROPHY COMPARED TO WHITE MATTER ATROPHY

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Background: Primary lateral sclerosis (PLS) is an idiopathic, neurodegenerative disorder of the upper motor neurons. The pathophysiology of PLS is still unknown. A number of small magnetic resonance studies have observed cortical atrophy, most notably in the cortical and subcortical portions of the precentral area. Atrophy of the motor cortex has been considered to be highly suggestive of PLS (1); however, not all studies have reported atrophy (2). This discrepancy may be due to the fact that most studies that have assessed atrophy have done so in a qualitative manner and thus only severe atrophy becomes apparent (3). In addition, it is unknown whether the atrophy is confined to the grey matter (GM), white matter (WM) or both.

Objectives: The aim of this study was to assess whether we could observe whole brain atrophy in patients with PLS and then to quantify atrophy and assess whether this was related to the loss of GM, of WM or both.

Methods: Patients with a clinically definite diagnosis of PLS were recruited from the neuromuscular clinic. High resolution T1-weighted images were acquired in patients with PLS and controls. SIENAX, a package for the estimation of atrophy at a single time-point, was used to calculate GM and WM volumes as well as whole brain volume (WBV). Mann-Whitney tests were used to compare the two groups for differences in age, GM and WM volume and WBV.

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Results: Eight patients and three controls have been scanned to date. There was no significant difference between the two groups' age: patients' mean age was 62.8 ± 10.7 years and controls' mean age was 55.67 ± 16.5 ($p=0.306$) years. There was a trend towards the mean WBV being smaller in patients than controls: 1.42 ± 0.09 vs. 1.51 ± 0.09, respectively ($p=0.133$). Mean GM was smaller in patients than in controls, 0.73 ± 0.07 vs. 0.80 ± 0.06, respectively ($p=0.194$). WM volume was similar in patients and controls, 0.68 ± 0.04 vs. 0.71 ± 0.4, respectively ($p=0.414$).

Conclusions: Our preliminary results suggest that there is whole brain atrophy in PLS and it is more closely related to GM atrophy than WM. These findings suggest that PLS pathology may be more closely related to cortical disease than the corticospinal tracts. WBV and more specifically GM volumes may help differentiate this disease from others.

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C48 VOLUMETRIC PROTON SPECTROSCOPIC IMAGING IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Previous MRS studies used either a single-voxel or a 2D-spectroscopic imaging (2D-SI) technique to detect cerebral metabolites in ALS patients. These techniques obtain metabolic information from a single voxel or multiple voxels in a slice. To understand the pathophysiology of the disease process, it is important to evaluate metabolic changes in the whole brain. This is the first study to use proton 3D-SI technique in ALS patients.

Objective: To assess the feasibility of a volumetric MRSI technique in detecting biomarkers for the upper motor neuron (UMN) dysfunction.

Methods: Eight patients with definite, sporadic ALS (54 ± 11 years of age) and six controls (48 ± 10 years) were scanned on a 3T MR scanner using a 3D-EPSI sequence (FOV: $280 \times 280 \times 180$ mm^3, $50 \times 50 \times 18$ phase encodes, slab thickness of 135 mm, TR/TE=1710/70 ms, and 26 minutes acquisition time). Severity of the disease was assessed using ALSFRS and vital capacity, and UMN dysfunction was evaluated by finger tap in 10 seconds and foot tap in 10 seconds. Spectroscopic data were processed using software developed in-house. Data from three ALS patients and one control were excluded due to poor quality. Data from eight regions of interest (precentral gyrus, corona radiata, internal capsule, midbrain and medulla) along the corticospinal tract were sampled to quantitate N-acetyl aspartate (NAA), creatine (Cr) and choline (Cho). Metabolite data were normalized. A two-tailed *t*-test was used and a *p*-value of <0.05 was considered significant.

Results: Important findings were: the normalized [NAA] was lower in patients with ALS compared to controls on both sides at the precentral gyrus (right: 28%, $p=0.04$; left: 20%, $p=0.06$), corona radiata (right: 22%, $p=0.05$; left: 16%, $p=0.04$) and internal capsule (right: 21%, $p=0.06$; left: 18%, $p=0.005$). The concentration of NAA did not reach a significant level at the left precentral gyrus and the right internal capsule but showed a trend towards it. There were correlations between the concentration of NAA at various levels along the motor pathway and the clinical measurements of UMN dysfunction ($r=0.4$–0.9). There were correlations between the ALSFRS and disease duration ($r=0.4$–0.5), vital capacity ($r=0.4$–0.5) and concentration of NAA ($r=0.4$–0.9).

Conclusions: The observed decrease in concentration of NAA and a trend for correlation with UMN dysfunction support previous findings that NAA may be used as a biomarker for UMN dysfunction. Our findings suggest that the whole-brain SI technique is well suited to measure the cerebral metabolite changes along the motor pathway in ALS patients.

Acknowledgement: Funding was provided by the Stanley Glaser Foundation Award.

C49 A COMPARISON OF CEREBRAL HAEMODYNAMIC PARAMETERS IN PATIENTS WITH ALS AND PLS ACCOMPANIED BY CHANGES IN COGNITIVE IMPAIRMENT

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Background: ALS and PLS are rare neurodegenerative diseases of the motor system. Increasingly, frontotemporal syndromes associated with cognitive impairment (CI) have been observed in ALS, often associated with concomitant decreases in brain perfusion. Although similar features have been observed in PLS, there is limited information contrasting ALS and PLS with respect to haemodynamic patterns in the presence or absence of CI.

Objectives: 1) To contrast cerebral haemodynamics in ALS and PLS patients with and without CI, and 2) to examine the relationship between CI and alterations in cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT).

Methods: The patient groups consisted of 12 ALS patients (seven men, five women) aged 40 to 63 years (mean, 51.4 ± 8.9 years), 18 PLS patients (nine men, nine women) aged 44 to 72 years (mean, 59.1 ± 8.7 years), and six spousal controls (five men, one woman) aged 34 to 63 years (mean, 52.3 ± 10.8 years). All patients and controls underwent NT followed by a computed tomography (CT) perfusion head scan to measure CBF, CBV, and MTT. Patients were stratified into two groups: 1) cognitively-intact PLS and ALS patients (PLS and ALS; defined as those patients having either one or no abnormal scores on NT testing) and 2) PLS and ALS patients with cognitive impairment (PLSci and ALSci; defined as those patients having two or more abnormal test scores). Abnormality of a test score was determined as a score that fell more than 2.0 SD below the mean. Stratified groups were compared with each other using a one-way ANOVA with post hoc Tukey tests to determine differences in haemodynamic parameters based on segmented regions of the brain corresponding to the four cortical lobes and deep grey matter structures. Results for the study were considered significant for $p < 0.05$.

Results: One of 12 ALS and four of 18 PLS patients were considered cognitively impaired based on NT. Since the ALSci group only had one member, statistical analysis could not be performed with this group. The ALS and PLS groups were statistically similar in all regions for haemodynamic parameters with the exception of CBV in the frontal and temporal grey matter, where PLS had an elevated CBV. The PLSci group had significantly increased MTT in all regions of the grey and white matter compared to the control and ALS groups. Furthermore, the PLSci group had significantly increased CBV in all grey matter regions (except the parietal lobe) compared with the controls and in the frontal and temporal region compared with the ALS group. Although the ALS group was statistically similar to controls, there was a global trend for increased MTT and CBV in the ALS group.

Conclusion: PLS and ALS were statistically similar, both demonstrating increased trends of CBV and MTT in all regions compared to controls. The PLSci group displayed the greatest deficits in cerebral haemodynamics, namely MTT.

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C50 THE PERFORMANCE OF CLINICAL OUTCOME MEASURES IN A PHASE II TRIAL OF GLATIRAMER ACETATE IN ALS

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Background: A standard set of clinical outcome measures, including the ALS Functional Rating Scale (ALSFRS-R), manual muscle testing (MMT), forced vital capacity (FVC) and quality of life scales (QOL), are currently applied in phase III trials in ALS. While test performance has been analysed extensively in large, long trials, how they perform in shorter trials with fewer subjects has not been well studied. As the number of neuroprotective agents increases, there is a growing need for small efficient phase II trials to screen drugs and to select the correct dose for efficacy trials. Which outcomes are best suited for small early phase trials is not yet known.

Objective: To assess the performance of clinical outcome measures from a short-duration small sample size phase II randomized controlled trial.

Methods: Data were analysed on the performance of the ALSFRS-R, MMT, FVC and QOL from a phase II trial of glatiramer acetate in 30 patients. Outcomes were obtained at baseline and at months 1–6. The rate of change in the ALSFRS-R at baseline (DeltaFS) was assessed to determine feasibility for stratification in future trials. Statistical comparisons were made using Pearson correlation coefficients, Kaplan-Meier life table curves, and Cox proportional hazards models.

Results: Correlations were found between ALSFRS-R/MMT ($r=0.60$, $p=0.0005$), ALSFRS-R/QOL ($r=0.50$, $p=0.0044$), and QOL/FVC ($r=0.42$, $p=0.0197$) at baseline. The slope of ALSFRS-R correlated with slopes of MMT ($r=0.73$, $p<0.0001$), FVC ($r=0.89$, $p<0.0001$), and QOL ($r=0.58$, $p=0.0007$). The slope of FVC correlated with slopes of MMT ($r=0.69$, $p<0.0001$) and QOL ($r=0.63$, $p=0.0002$). Baseline ALSFRS-R ($p=0.0490$) and FVC ($p=0.0005$) predicted survival, while MMT did not ($p=0.3987$). Slopes of ALSRS-R ($p=0.0028$) and FVC ($p=0.0030$) were also found to predict survival. When dichotomized by baseline median scores, the DeltaFS ($p=0.0235$, median=0.5) and ALSFRS-R ($p=0.0037$, median=33) were associated with survival. The DeltaFS, when broken down by tertile, was also associated with survival ($p=0.0063$).

Conclusions: Standard clinical outcomes performed reliably in a short-duration small sample size trial. The ALSFRS-R baseline score and slope predicted survival. MMT and FVC, while reliable, were less clearly predictable in rates of decline and were less strongly predictive of survival. The ALSFRS-R, because of its simplicity and reliability may be best suited for small clinical trials, and the rate of decline taken at baseline visit (DeltaFS) could be used to stratify patients by rate of progression. The efficiency of early phase clinical trials might be improved by using only one or a few highly reliable and meaningful outcome measures, such as the ALSFRS-R.

C51 IDENTIFYING RESPONDERS TO INCREASE POWER AND SENSITIVITY IN ALS CLINICAL TRIALS

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Background: Variability in patient rates of decline supports the hypothesis that ALS is a heterogeneous

disease. This suggests that it may be unrealistic to expect that a single agent will be uniformly effective for all patients. It would be desirable to define a statistical test that can detect responses in a subset of patients while other patients show no response.

Objective: To compare power of an analytic plan designed to detect a subset of responding patients with two conventional comparisons based on responses of all patients.

Methods: We used a linear mixed effects model to estimate pre- and post-treatment changes in ALSFRS slope for each patient in two phase III negative trials of gabapentin and TCH346. These results were used to set a cut-off point for defining response to treatment based on the change in slope. A test for treatment effect is based on counting the number of responders (i.e. those with changes greater than the cut-off point) in treated compared to placebo arms of a clinical trial. Sample sizes for this test (Responder Test) were compared with those required for two conventional tests: a *t*-test, comparing mean responses, and a non-parametric test (Wilcoxon test) for all patients. Sample sizes for the Responder and Wilcoxon tests were obtained from tables in a publication describing rank tests designed for small proportions of responders (1).

Results: Estimates for initial slope and change in slope of ALSFRS after four months in the clinical trials were similar. One hundred and sixty-six patients from a gabapentin trial (where treatment had no effect) and 110 placebo patients from a TCH346 trial had six or more monthly ALSFRS scores. Mean slopes during the initial four months were: 0.75(SD 0.78) units/month for gabapentin and −0.74(1.06) for TCH346 placebo. Mean slope changes after the first four months were −0.04(1.22) and −0.14(1.18), respectively. Subsets of patients in the gabapentin trial (22%) and in the TCH346 trial (23%) were fast progressors with initial slopes greater than −1.2 units/month. A slope change of 1 SD in either study would represent a slope reduction of ~1.2 ALSFRS units/month, which might be more readily seen in the fast progressor subset. Slope changes of this magnitude or greater were seen in 13% of gabapentin and in 11% of TCH346 patients. Sample sizes for change of 1 or 2 SD and different fractions of responders are compared below:
Sample sizes (per arm) required for 80% power:

Responder increase (SD units)	Responding fraction	*t*-test of means[a]	Wilcoxon test[b]	Responder test[b]
1	0.1	1348	850	482
1	0.2	359	290	169
1	0.3	167	132	95
2	0.1	576	274	129
2	0.2	205	103	54
2	0.3	115	50	35

[a]Based on *t*-test with mean and SD calculated for a mixture distribution [b]Based on extrapolations from Table IV in the reference (1).

Conclusions: A phase III clinical trial examining responders with 1–2 unit reduction in ALSFRS slope could result in smaller sample size and more efficient and sensitive trials.

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SESSION 8A NEUROTROPHIC FACTORS

C52 HEPATOCYTE GROWTH FACTOR (HGF) AS A NOVEL NEUROTROPHIC FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Hepatocyte growth factor (HGF) was first identified as a potent mitogen for mature hepatocytes and was molecularly cloned in 1989 (Nakamura et al., BBRC, 1984; Nakamura et al., Nature, 1989). HGF prevents endotoxin induced lethal hepatic failure in mice with fulminant hepatitis via its anti-apoptotic activity, and HGF gene therapy is capable of improving the survival rate of rats with lethal liver cirrhosis (Kosai et al., Hepatology, 1999; Ueki et al., Nat Med, 1999). In addition to its role as a hepatotrophic factor, extensive expression and functional studies, including knock-out/knock-in mouse strategies, revealed HGF to be a novel neurotrophic factor for a variety of neurons (Honda et al., Mol Brain Res, 1995; Maina et al., Nat Neurosci, 1999). Furthermore, HGF is one of the most potent survival-promoting factors for motor neurons. We show that overexpression of HGF in the nervous system attenuates motor neuron death and axonal degeneration, improves motor function and prolongs the life span of transgenic mice overexpressing mutated Cu2+/Zn2+ SOD1. HGF prevented induction of proapoptotic proteins (various types of activated caspases) in both spinal and brainstem motor neurons and increased X-linked inhibitor of apoptosis protein (XIAP) in these motor neurons. In addition, HGF retained the levels of the glial-specific glutamate transporter (EAAT2/GLT1) in reactive astrocytes, presumably favoring a reduction in glutamatergic neurotoxicity. We also present the efficient activation of c-Met/HGF receptor by HGF in an ALS-dependent fashion. Taken together with the evidence that HGF and c-Met are regulated in the spinal cord of both familiar and sporadic patients with ALS in a manner similar to the transgenic ALS model, we propose that local application of HGF may be a safe and effective therapeutic for patients with ALS.

C53 INTRATHECAL DELIVERY OF HEPATOCYTE GROWTH FACTOR AT THE ONSET OF PARALYSIS SLOWS DISEASE PROGRESSION IN A RAT MODEL OF ALS

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Background and objectives: We developed a rat model of ALS expressing a human SOD1 transgene with two ALS-associated mutations, G93A and H46R. Some experimental manipulations are difficult in Tg mice because of size limitations. However, this Tg rat model allows routine implantation of infusion pumps for intrathecal drug delivery.

Hepatocyte growth factor (HGF) is one of the most potent survival-promoting factors for motor neurons. We reported that introduction of the HGF gene into neurons of G93A Tg mice attenuates motor neuron degeneration and increases the lifespan of these mice. Thus, HGF is a good candidate agent for treatment of ALS. Currently, treatment using recombinant protein is closer to clinical application than gene therapy. Therefore, we examined continuous intrathecal delivery of human recombinant HGF (hrHGF) into Tg rats using implanted infusion pumps for selective and less invasive supply of HGF to the spinal cord.

Methods: To examine the motor neuron protective effect and therapeutic potential of hrHGF, we administered 40 and 200 μg of hrHGF or vehicle alone to 100-day-old G93A Tg rats around the age that pathological changes of the spinal cord appear but the rats do not show weakness clinically. 200 μg of hrHGF or vehicle alone was administered to 115-day-old G93A Tg rats at the time of onset of paralysis. HrHGF was administered for four weeks in each case.

Results: *Administration of hrHGF to 100-day-old G93A Tg rats for four weeks.* At 130 days old, the average numbers of motor neurons in the ventral horn were: non-Tg rats, 19.2 ± 3.3; vehicle only, 2.9 ± 1.3; 40 μg hrHGF, 6.3 ± 2.1; 200 μg hrHGF, 11.2 ± 4.2. Significantly more motor neurons survived in hrHGF-treated (40 μg, $p < 0.01$; 200 μg, $p < 0.001$) than in vehicle-treated G93A

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Tg rats. hrHGF prevented motor neuron death in G93A Tg rats in a dose-dependent manner.

Administration of hrHGF to 115-day-old G93A Tg rats for four weeks. There were no statistically significant differences in the onset between the groups. In contrast, 200 μg hrHGF extended mean survival by 11 days compared to vehicle-treated G93A Tg rats ($p=0.0135$), although G93A Tg rats show very rapid disease progression and die within 20 days of disease onset. The average periods from the onset to death were 16.9 ± 8.17 and 27.5 ± 11.1 days in vehicle ($n=8$) and hrHGF ($n=8$) groups, respectively. The latter represented an increase of 63% relative to vehicle-treated controls even started at the ALS onset.

Discussion and conclusions: The intrathecal administration of hrHGF attenuated motor neuron degeneration, and prolonged the duration of the disease by 63% even when administered from the onset of paralysis. We claim therapeutic effects of continuous intrathecal administration of hrHGF in Tg rats. The results should prompt further clinical trials in ALS using continuous intrathecal administration of hrHGF.

C54 INTRACEREBELLAR INJECTION OF AAV-IGF-1 IMPROVES MOTOR FUNCTION AND EXTENDS SURVIVAL 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 loss of motor neurons in the cortex, brainstem and spinal cord. While there are numerous hypotheses for disease onset and progression, neurotrophic factors such as insulin-like growth factor 1 (IGF-1) have shown promise in delaying motor neuron death in ALS. Several studies have shown the benefit of using muscle-specific IGF-1 that have led to significant life extension and improved motor function in ALS transgenic mice. An important question raised by these studies is whether IGF-1's mode of action was on the muscle, the neuromuscular junction or the motor neuron cell body. It is also becoming increasingly clear that multiple cell types other than motor neurons contribute to the pathogenesis of ALS. This raises the question as to whether potential therapies should be directed not only to motor neurons, but also to the surrounding cellular environment for maximal therapeutic efficiency.

Objectives: We sought to determine the effects of CNS delivered IGF-1 without targeting the muscle and neuromuscular junction. We accomplished this by delivering IGF-1 expressing AAV vectors (that are capable of retrograde transport) to a region of the brain that has extensive connections with the brainstem and spinal cord – the deep cerebellar nuclei (DCN) of the cerebellum. By targeting the DCN we also tested whether IGF-1 expression by cells that comprise a motor neuron's surrounding cellular environment is sufficient to modify disease progression in SOD1 mice.

Methods: In this experiment we evaluated the efficacy of bilateral delivery of AAV1–IGF-1 and AAV2-IGF-1 to the DCN in symptomatic SOD1G93A mice. Starting at 80 days of age, SOD1G93A mice underwent rotarod and grip strength testing to assess motor function. At 90 days of age mice received stereotaxic injections of AAV1-IGF-1 ($n=26$), AAV2-IGF-1 ($n=27$), AAV1-GFP ($n=26$) or AAV2-GFP ($n=25$) aimed at the DCN.

Results: We found that AAV-IGF-1 treatment (regardless of serotype) significantly reduced gliosis throughout the brainstem and spinal cord, promoted motor neuron survival, improved motor performance in both rotarod and grip strength tests and significantly extended lifespan. IGF-1 expression was detected throughout the brainstem and spinal cord using PCR and ELISA. Treatment with AAV-GFP had no effect on any of the parameters mentioned above. Positive GFP fibers and/or cell bodies were seen in the cortex, brainstem (i.e. motor trigeminal nucleus, hypoglossal nucleus and facial nucleus) and in each division of the spinal cord.

Conclusion: Our results indicate that direct administration of AAV-IGF-1 into the DCN modifies disease progression in a mouse model of ALS.

C55 CHARACTERIZATION OF COLIVELIN-MEDIATED NEUROPROTECTION AGAINST ALS-RELEVANT INSULTS

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Background: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. Currently, there is no cure for ALS. We have previously reported that intracerebroventricular (i.c.v.) injection of Colivelin, a hybrid peptide consisting of activity-dependent neurotrophic factor (ADNF) and a potent humanin (HN) derivative termed AGA-(C8R)HNG17, improves motor performance and prolonged survival of G93A-SOD1 transgenic (Tg) mice (1–3).

Objectives: To determine the neuroprotective mechanism of Colivelin, we compared the effect of ADNF, AGA-(C8R)HNG17, or Colivelin on motor performance and the lifespan of G93A-SOD1 Tg mice *in vivo* and on death of NSC34 motor neuronal cells overexpressing G93A-SOD1- *in vitro*.

Methods: G93A-SOD1 Tg mice, implanted with a cannula, were i.c.v. injected with 10 pmol/mouse of ADNF, AGA-(C8R)HNG17 or Colivelin every other day from the age of 80 days. Motor performance was evaluated in the rotarod test performed every three days and mean ages of death were compared among the groups. NSC34 cells were transfected with G93A-SOD1 cDNA, together with or without dominant negative forms (dn) of Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) or STAT3, by lipofection and cultured in the presence or absence of the peptides at 100 nM. Seventy-two hours after transfection, cell mortality was measured by WST-8 assay.

Results: Mean survivals of control (vehicle)-, ADNF-, AGA-(C8R)HNG17-, and Colivelin-treated mice were 142.0 ± 2.2, 143.6 ± 1.7, 147.1 ± 1.8, and 151.5 ± 1.8 days, respectively. Colivelin significantly prolonged the life span vs. control ($p=0.0009$), while neither ADNF nor AGA-(C8R)HNG17 treatment did so. Death induced by G93A-SOD1 was completely suppressed by 100 pM ADNF, AGA-(C8R)HNG17, or Colivelin. Neuroprotective effect of ADNF was antagonized by dominant-negative (dn) CaMKIV, while that of AGA-(C8R)HNG17 was antagonized by dnSTAT3. Colivelin-mediated neuroprotection was antagonized only when both dnCaMKIV and dnSTAT3 were simultaneously expressed.

Discussion and conclusions: Colivelin significantly prolonged survival of ALS mice, while administration of the same amount of ADNF or AGA-(C8R)HNG17 did not, indicating that fusion of the two peptides gives rise to an additional advantage in neuroprotection *in vivo*. Colivelin protected NSC34 cells from death induced by overexpression of G93A-SOD1 via activating CaMKIV and STAT3 *in vitro*. Thus, we conclude that Colivelin is a promising neurotrophic factor for treatment of ALS.

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C56 NGR ANTAGONIZES P75NTR-DEPENDENT MOTOR NEURON DEATH: IMPLICATIONS FOR ALS

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Background: Spinal motor neurons express the neurotrophin receptor p75NTR during the embryonic period of naturally occurring cell death but this expression gradually ends after birth. Adult motor neurons, however, can re-express p75NTR following axotomy or in ALS, which has been involved in triggering the motor neuron death characteristic of these conditions. Indeed, our recent *in vitro* findings revealed that NGF secreted by reactive astrocytes as well as spinal cord lysates from symptomatic ALS SOD1(G93A) mice stimulate motor neuron death. On the other hand, growing evidence shows that p75NTR also interacts with the glycosyl-phosphatidyl-inositol-anchored central nervous system Nogo-66 receptor NgR to inhibit axon repair after spinal cord lesion. Therefore, p75NTR is able to trigger either neuronal death, upon binding to neurotrophins such as NGF and their precursor forms, or neurite outgrowth inhibition when bound to NgR; albeit, the crosstalk between these two pathways has not been explored to date.

Objectives: In the present study, we asked whether NgR activation, via binding to specific ligands, could regulate motor neuron survival by modulating p75NTR-induced cell death under conditions in which both receptor partners appear coexpressed.

Methods: To assess whether previously characterized NgR ligands, Pep4 and NEP1-40, can affect motor neuron survival, we administered these ligands to embryonic rat motor neuron cultures challenged with NGF in the presence of a low steady state concentration of nitric oxide or cocultured on reactive astrocytes secreting NGF. We also evaluated the ability of both NgR ligands to modulate motor neuron death induced by spinal cord lysates from symptomatic SOD1(G93A) mice. Finally, we tested whether Pep4 and NEP1-40 were able to promote motor neuron survival *in vivo* following neonatal sciatic nerve axotomy.

Results: Pep4 and NEP1-40 counteracted the death action of p75NTR in the various *in vitro* experimental paradigms used in this study. Surprisingly, they exhibited similar protection against p75NTR-mediated cell death, which clearly contrasts with their antagonistic effects on neurite outgrowth. Most importantly, both NgR ligands

abolished post-axotomy ipsilateral motor neuron loss compared to vehicle-treated animals.

Conclusion: The results of this study show for the first time that Pep4 and NEP1-40, two NgR ligands typically reported as regulators of neurite outgrowth, are able to promote motor neuron survival *in vitro* and *in vivo*. We therefore describe an as yet unknown function of NgR in maintaining neuronal survival against the death-promoting effect of p75NTR. These findings may have relevance when addressing future strategies to protect motor neurons from ALS.

C57 VEGF RESCUES THE LOSS OF ACTIVATED AKT PRECEDING MOTOR NEURON DEGENERATION IN ALS

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Background: Amyotrophic lateral sclerosis is one of the most devastating neurodegenerative disorders. The pathogenesis of this disease that results in a relentless loss of motor function remains unknown. We recently reported that treatment of mutant $SOD1^{G93A}$ overexpressing rats with VEGF resulted in a significant increase in survival compared to the control group (1). The precise mechanism of the neuroprotective effect of VEGF in ALS remains to be elucidated, but modulation of the phosphoinositol-3-kinase/Akt cascade is one of the most likely targets.

Objectives: In the present study, we aimed to investigate the pathophysiological role of Akt in ALS and the influence of VEGF on this important anti-apoptotic protein.
Methods and results: Immunohistochemical studies showed that motor neurons of both sporadic, familial SOD1 and familial non-SOD1 ALS patients lack phospho-Akt, compared to control patients. In mutant $SOD1^{G93A}$ overexpressing mice, Western blot analysis showed that activated protein kinase B or phospho-Akt is indeed lost very early in the disease. Immunohistological experiments showed that this loss is confined to motor neurons, supporting the pathogenetic relevance of the potent anti-apoptotic kinase Akt. *In vitro* transfection experiments with constitutively active Akt revealed the neuroprotective effect of Akt in mutant dependent cyclosporin A-induced cell death in $SOD1^{G37R}$ overexpressing N2A cells. To evaluate the relevance of the Akt pathway in the neuroprotective effect of VEGF, Western blot analysis was performed in spinal cord homogenates of VEGF-treated $SOD1^{G93A}$ rats. These results showed that treatment with VEGF resulted in a significant increase in the levels of phospho-Akt.

Conclusions: Our data support the relevance of Akt to the selective loss of motor neurons in ALS and provide evidence for its role as a target of VEGF. Therefore, further development of therapeutic strategies targeting the phosphoinositol-3-kinase/Akt pathway may be useful.

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SESSION 8B INTERNATIONAL PERSPECTIVES ON CARE AND QUALITY OF LIFE

C58 INDIVIDUAL ALS CARE IN THE JAPANESE 'NANBYO' CARE MODEL: COMPARISON WITH PALLIATIVE CARE APPROACHES IN ACHIEVING BEST QUALITY OF LIFE

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The concept of 'nanbyo' in Japan can be traced to the administrative measures of the Japanese Ministry of Health, Labor and Welfare (MHLW) in 1972. Nanbyo (meaning, nan: difficult and worried, byo: illness in Japanese) refers to diseases that are incurable and intractable in physical and psychosocial aspects. Three pillars of the measures, including research programmes on nanbyo, a care system for patients with nanbyo, and reimbursement of medical expenses to individual patients with nanbyo started in 1972. At the beginning, the administrative definition of nanbyo included Behçet's disease, myasthenia gravis, systemic lupus erythematosus, cancer in children, nephritis in children, bronchial asthma in children, hemodialysis patients, severe physical and mental disability in children, and progressive muscular dystrophy; ALS was added in 1974 according to the annual revision.

At present, funding by MHLW for nanbyo researchers provides 2 billion yen for 63 research groups. Each has missions to survey and study its own focused diseases including ALS. Clinical data including history, signs and symptoms, treatments, and activities of daily living are collected in exchange for reimbursement of medical expenses. This accounts for 70 billion yen.

Research and clinical practice in ALS have made dramatic progress under the measures of MHLW since 1974. In the early1980s, a small number of ALS patients started tracheostomy ventilation in hospital. Multi- and inter-disciplinary care is thought to be necessary for improving quality of life of patients with ALS. Although the nanbyo care program together with the elderly care insurance system and disabled patients' support system worked very well, it was not enough for the patients and their family in reality, and their burden was apparently heavy. Even in this care environment some patients with ALS and their families published books or articles about their happy lives with ventilators. Many ALS patients were encouraged to live longer with a ventilator. In the mid-1990s home mechanical ventilation started to be officially supported by Japanese health insurance. Relatively new techniques such as respiratory physiotherapy, mechanically assisted coughing, and PEG gradually became available in ALS care in Japan.

Although it may be valuable that non-invasive ventilation and tracheotomy ventilation are both available in Japanese ALS care, psychological and spiritual conflicts sometimes occur in patients and their families in the area of informed consent to each palliation. It may now be possible that using individual QOL assessment methods such as SEIQoL and a spiritual care approach derived from UK hospice care will help diminish these conflicts. Although ALS is still incurable and intractable, patients living with ALS can sometimes establish a new meaning of life and can find new perspectives of life. We think this may be one of the goals of ALS care.

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C59 WHAT CAN WE LEARN ABOUT LIVING WITH ALS/MND FROM PERSONAL ILLNESS NARRATIVES POSTED ON THE INTERNET?

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Background: Experiences reported in internet narratives, as opposed to those appearing in other media, are more likely to be written as they occur and rarely undergo reflective editorial processes. Hence, they are often written with a degree of poignancy and intensity not found in other media.

Objectives: To determine: 1) What PALS/PwMND write about their illness experiences; 2) Why PALS/PwMND write about their illness experiences; 3) What the benefits of writing are.

Methods: The process of identifying internet narratives by people with ALS/MND has been reported previously (1). This process ultimately generated a sample of 64 electronic narratives, which were subjected to a content and thematic analysis, aided by Nvivo software.

Results: A number of key themes emerged. Most narratives contain a strong emphasis on regular condition updates, often including tips on how to manage particular symptoms and disabilities. Several authors, despite increasing loss of function, stress the importance of what can still be done, rather than what has been lost. Many refer to this as ''living with, not dying of ALS/MND''. There is a sense that authors have remained 'whole' in their thoughts, despite their physical deterioration. Frequent comments are made about the impact on and changes to relationships with others, usually partners/spouses and referred to as their ''loss of life''. There are numerous reports of searches for alternative and complementary therapies, which help to provide a sense of personal control in a life that is otherwise ''out of control''

and generates a feeling that they are doing something for themselves when conventional medicine has little to offer. Most authors display altruistic motivation for writing; they share their experiences (including the testing of unproven treatments) and knowledge of the disease to lessen the burden on others. They provide resources for others, in terms of advocacy, information and support; several raise awareness and educate, many inspire, offer hope and provide an example to other sufferers of how to cope with the diagnosis of ALS/MND.

Authors view the narrative writing process as therapeutic, and for some, helping to remove the final barrier to admitting their diagnosis by ''going public''. Readers report being comforted, supported and enlightened. Publishing on the internet provides an opportunity to communicate with other PALS and reduce isolation. By being visible, authors act as a beacon for others and a source of invaluable information for patients, carers and health professionals.

Discussion and conclusions: Illness narratives enable individuals to document how illness has impacted on their lives and the lives of those closest to them. The electronic narratives of illness identified in this study comprise a rich source of data about issues which people with ALS/MND regard as important.

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C60 HOUSE CALLS: MULTI-DISCIPLINARY OUTREACH MEDICINE FOR THE HOME BOUND ALS PATIENT

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Background: As ALS progresses, activities of daily living become more difficult for patients to perform. Furthermore, advanced disease often renders the ALS patient unable to access medical therapy, participate in clinical trials, and receive the support of a multi-disciplinary team. Currently, home health services are not available to most patients. If available, these services are usually limited to restricted nursing care and/or therapy services and do not adequately meet the needs of the homebound ALS patient.

Objective: To assess the feasibility of implementing multi-disciplinary outreach care to homebound ALS patients in order to provide a continuum of care for the duration of the disease.

Methods: The ALS Home Outreach Team consists of a neurologist, physical therapist, social worker, and a patient services coordinator from the Muscular Dystrophy Association. Patients are visited every three to six months and each visit lasts approximately one to two hours. The neurologist performs a full physical/neurological examination, assesses respiratory and nutritional status, and updates prescriptions. The physical therapist assesses the patient's functional status, updates the home exercise program, performs family and/or caretaker education, and assesses equipment utilization and needs. The social worker assesses the patient's current financial and insurance status, advises on changes in disability or insurance policies, and performs patient and family counselling as needed. The patient services coordinator facilitates obtaining needed equipment and/or other services that are identified during the visit.

Results: Multiple benefits of the House Calls Program have been identified since its inception. The medical care team is updated on the patient's status such that they feel comfortable discussing medical needs over the phone and/or refilling prescriptions even though the patient has not been in the clinic for many months. The medical team also has the benefit of assessing patients in their home environment where the logistics of day-to-day activities can accurately be addressed. Patients have a full medical examination and assessment without having to leave their home and are able to maintain a connection to their medical team. Required follow-up visits from study protocols can be performed, thus minimizing drop-out rates. Caretakers receive in-home support and education and have the opportunity to problem-solve difficult tasks.

Discussion: Our experience suggests that multi-disciplinary 'house call' care is successful as a mechanism to maintain a continuum of care when patients are no longer able to leave their homes for medical care. Future benefits with this intervention include reduced hospitalizations, increased completion rates for required study visits, increased compliance with equipment/exercise recommendations, and improvements in quality of life for homebound patients and their caretakers.

C61 A STUDY OF COVARIATES OF FATIGUE IN ALS

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Background: The symptom of fatigue has been recognized in the ALS population. However, the relationship between fatigue and disease severity or other clinical features has not been well characterized.

Objective: To determine the relationship between fatigue in ALS and other disease variables and covariates of survival.

Methods: We performed a cross-sectional study of probable or definite ALS patients compared to normal, non-caregiver controls. We measured fatigue with the Multidimensional Fatigue Inventory-SF (MFSI-SF), depression with the Beck Depression Inventory (BDI), hypersomnolence with the Epworth Sleepiness Scale (ESS), and quality of life with the McGill Quality Life Scale. Regression and χ^2 analyses were performed to study

the relationship between fatigue and disability, progression, FVC, frontal lobe function, and comorbidity/medication use. Statistical significance was set at $p \leqslant 0.05$. Results are presented as mean $\pm$ SD.

Results: We evaluated 52 ALS patients (ALS) and 32 non-caregiver, normal controls (NC). Measures of fatigue (ALS: 29 ± 16 vs. NC: 6.4 ± 6.6; $p < 0.0001$) and depression (ALS: 12 ± 8.6 vs. NC: 5.0 ± 8.6; $p < 0.01$) were significantly elevated above controls, with prevalence of 80% and 26%, respectively. Quality of life scores were lower (ALS: 7.1 ± 1.0 vs. NC: 8.4 ± 1.4; $p < 0.01$). ALS patients showed a strong trend toward increased hypersomnolence ($p = 0.059$). Fatigue severity showed a significant relationship with disability as measured by the Appel ALS score ($R = 0.4$), but not with disease progression ($R = 0.09$). There was a trend toward a significance with FVC ($R = -0.24$). Fatigue showed the strongest correlation with depression ($R = 0.74$) and an inverse relationship with quality of life ($R = -0.54$). In the majority of cases subjects who scored within the normal range on the BDI, MFSI-SF (fatigue) scores were still significantly higher in the ALS patients ($p < 0.001$). There was no relationship with comorbidities or concomitant medications, although there was trend toward significance for frontal lobe dysfunction and riluzole therapy.

Conclusions: Fatigue is highly prevalent in the ALS population and its presence appears to be independent of disease progression and disability. Its high prevalence in patients without depression suggests that fatigue may also be independent of such non-motor symptoms. Future studies will be directed at investigating other potential variables for fatigue, including frontal lobe dysfunction, respiratory function, and riluzole therapy which all showed an association trend towards significance in this study.

C62 INTRATHECAL BACLOFEN PUMP FOR TREATMENT OF MEDICALLY REFRACTORY RIGIDITY AND SPASTICITY IN PATIENTS WITH MOTOR NEURON DISEASE

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Background: The medical management of rigidity and spasticity is often incomplete due to intolerance to one or more of the currently available medications. Most commonly oral antispasmodic medications such as baclofen, dantrolene and tizanidine result in fatigue or perceived weakness and these symptoms may offset a gain in functional ability.

Objective: We report here the experience of 20 consecutive patients with motor neuron disease, in our clinic, who received intrathecal (IT) baclofen as an alternative to medically refractory rigidity and/or spasticity.

Results: All patients who received the IT baclofen pump were resistant or intolerant to at least two available medications. Ten of the 20 patients we implanted had tried three medications without clinical benefit at maximum tolerated doses. Among the 20 patients we report here, one patient was unable to tolerate any dose of baclofen, 19 patients reached their maximum tolerated dose without a significant benefit and two of those patients received no benefit at all. Sixteen patients received dantrolene, with three unable to tolerate the medication and 13 unable to achieve a satisfactory benefit from maximum tolerated dose of dantrolene. Twelve patients were treated with tizanidine, five received no benefit, two were unable to tolerate it, and four were receiving suboptimal benefit at maximum tolerated dose.

Patients received the intrathecal baclofen pump with initial dosing of 50 mcg per 24 h period. This dose was increased incrementally until the maximal benefit was reached. The maximum dose adjustment we used was 50 mcg per titration step. The optimal therapeutic dose of IT baclofen varied greatly by patient (75 mcg to 1450 mcg daily). Intolerance to oral baclofen was not a predictor of IT dose or tolerance. IT dose delivery protocol also varied among our patients. Continuous steady delivery of the drug throughout the day was optimal in 65% of the patients, while periodic bolus delivery was needed in 35% of the implanted patients.

Oral anti-spasmodic medication was used by seven patients after IT pump placement to achieve optimal benefit; however, significantly lower doses were used and side-effects were not problematic. Most patients noted a decrease in fatigue and sedation with the decrease in oral medications. One patient noted an initial benefit from the IT pump, but did not note a long-term benefit. No patients experienced complications from the surgery.

Conclusions: Clinical improvement (less rigidity and/or spasticity) was measurable in all patients implanted. Subjective improvement was reported in 90% (18/20) patients by 3.3 months (4–365 days) after implantation. Overall, the IT delivery of baclofen via surgically implanted intra-abdominal pump was well tolerated and beneficial to most refractory patients with rigidity and spasticity. Clinical benefit was usually realized by three months after implantation. This procedure should be considered as an aggressive treatment option in the appropriate clinical setting.

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SESSION 8C EPIDEMIOLOGY AND GENETICS

C63 OCCUPATIONAL RISK FACTORS IN SPORADIC ALS: A SYSTEMATIC REVIEW

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Background: A large number and variety of studies have been performed in the search for exogenous risk factors in sporadic ALS, often producing conflicting results. Most reviews of these studies, however, are narrative and few have used a systematic approach in defining inclusion criteria and comparing study methodology.

Objective: To perform a systematic review of occupational risk factors in sporadic ALS.

Methods: An extensive search strategy was designed and run in the Medline, Embase, Cinahl, and Cochrane databases. A study was included if the following criteria were met: it had to 1) be a case control or cohort study; 2) examine occupations in patients with sporadic ALS and a reference group; 3) not be performed in study populations in Guam, the Kii Peninsula or other endemic areas. Included studies were appraised according to a classification of evidence developed for risk factors in ALS by Armon. Each occupation studied was grouped according to the International Standard Classification of Occupations (ISCO-88) and for each occupational category available study findings were summarized.

Results: The search strategy provided 3773 potentially relevant studies. After screening of title, abstract, and full-text articles according to the selection criteria, 56 studies were included: three cohort, 24 case-control, 16 mortality cohort, and 13 mortality case-control studies. Studies making use of mortality registers were grouped separately. Most of these studies fell into Armon's level of evidence class III or IV. One hundred and thirty different major and minor occupational groups were examined in one or more studies. Studies differed with regard to design, the types of measures of association computed, and the occupational categories studied. Because of this heterogeneity, quantitative pooling per occupational group was impossible. To summarize the data, the vote counting method was applied. A minority of the occupations studied had a positive vote count. These were power plant operators, veterinarians, health associate professionals, athletes, hairdressers, and potters.

Conclusion: Multiple studies were performed for individual occupations, often giving conflicting results. The methodological quality of the studies did not exceed Armon's level of evidence class III. Most studies did not fall into higher categories because of the testing of multiple hypotheses, the presence of small numbers of individuals with ALS with a specific occupational exposure, and selection bias. Well-designed large prospective cohort studies are needed focusing on a few occupations. Bearing the limitations of study quality in mind, this review concludes that the most promising occupations are power plant operators, veterinarians, health associate professionals, athletes, hairdressers, and potters. Unfortunately, there is no common underlying mechanism for developing ALS that can be explained by these occupations. A job-exposure matrix can elucidate specific occupational exposures and be utilized in future studies.

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C64 INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN LOMBARDY, ITALY

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Background: The incidence and trends of amyotrophic lateral sclerosis (ALS) have been mostly assessed in small population samples. Data from well-defined large populations at risk are limited.

Objective: To define the incidence, time and geographic trends of ALS in Lombardy, northern Italy, through a population-based register.

Methods: This study was performed in nine provinces of Lombardy (population 4,947,554). Patients with newly diagnosed ALS were enrolled during the calendar years 1998–2002. The main source of cases was a prospective regional register of ALS, started on 1 January 1998. The register was based on a network of caring neurologists from neurological, neurophysiological and rehabilitation units in the study area. Cases were also traced through the Hospital Discharge Diagnosis (HDD) code 335.2 (MND) at the Central Regional Archive.

For each eligible patient, the caring physician was required to collect the main demographic and clinical information in a semi-structured form. A panel of experts reviewed all forms and each eligible patient was classified as definite, probable, probable - laboratory supported, possible or suspected ALS, according to a combination of the original and revised El Escorial diagnostic criteria. Overall crude, age and sex-adjusted mean annual incidence rates were calculated for the entire study population and for each year and province separately. Age specific incidence rates were also provided. Age- and sex-adjustment was made with the direct method

of standardization using the 2001 Italian population for reference.

Results: During the study period we collected data on a total of 517 patients (293 male, 224 female; male:female ratio 1.3) aged 18–92 years at diagnosis (mean 63.6 years). Onset of symptoms was bulbar in 29% of cases. ALS was definite in 45%, probable in 27%, probable - laboratory supported in 3.5%, possible in 15%, and suspected in 10% of cases. Mean disease duration at diagnosis was 10.6 months. The standardized incidence ratio was 2.10 per 1,000,000/year (95% CI 1.19–3.19). The ratio was 2.43 in men and 1.76 in women, tended to increase with age up to the years 70–74, and to decrease thereafter. The rate was virtually unchanged in each year of the study period and presented moderate variations when comparing the different provinces. The incidence of definite ALS was 0.93 per 100,000. The incidence of spinal-onset ALS was 1.35 and that of bulbar-onset ALS was 0.60. The rate was consistently higher in men with spinal-onset ALS, peaking at age 75–79 years compared to men with bulbar-onset ALS and women. In the latter, the rate tended to overlap when comparing spinal-onset to bulbar-onset ALS. The disease peaked at age 70–74 years in women and fluctuated at age 65–69 years through age 80–84 years in men with bulbar-onset ALS.

Discussion and conclusions: This study confirms a steady incidence ratio of ALS over a five-year period, with no evidence of significant temporal and geographic clusters. The age-specific incidence of the disease tends to vary according to the site of onset.

C65 A META-ANALYSIS OF THE INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN EUROPE: THE EURALS COLLABORATIVE STUDY

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Background: Recent population-based prospective studies have shown that the incidence of ALS is uniform across Europe. However, a number of important questions remain unanswered: Does the incidence of ALS continue to increase among the very elderly? Is the risk of developing ALS in females approaching that of males? Existing registers are unable to definitively answer these questions due to their limited catchment populations of approximately 4 million each. EURALS is a collaborative effort designed to unify the six existing European ALS registers and to provide useful epidemiological data for a large population across Europe. We present the results of a meta-analysis of the epidemiology of ALS in a cohort of 17 million for the years 1998 and 1999.

Objectives: 1) To determine the incidence and distribution of bulbar and spinal subtypes of ALS across Europe; 2) to investigate if the incidence of ALS continues to increase among the very elderly age groups (i.e. 75 years old, plus); 3) to evaluate if the risk of ALS is increasing among women compared to men.

Methods: Incidence data for the time period 1 January 1998 to 31 December 1999 were available from six studies carried out in three European countries, three from Italy (Piemonte, Lombardia, Puglia), one, respectively, from Scotland, England (Lancashire), and Ireland. The diagnosis of ALS was based on the first version of the El Escorial criteria (EEC). These data were pooled and age- and sex-specific incidence rates were calculated as the number of new cases divided by the person-years at risk. Population denominators were obtained from local government censuses.

Results: Data were available from 34,167,244 person-years representing a population of 17,083,622 (Ireland 3,703,100, Piemonte 4,407,782, Lombardia 4,947,554, Puglia 4,025,186). Six hundred and ninety-seven incident cases were diagnosed during the two year study period 1998–1999 (317 females (45.5 %) and 380 males (54.5%); 361 cases in 1998 and 336 cases in 1999). The crude incidence of ALS was 2.04 per 100,000 person-years. Nearly two-thirds of cases were of spinal onset (65.9%), the remainder being classified as bulbar onset (29.1%), generalized onset (4.3%) and unspecified (0.7%). Men had a higher incidence of ALS than women during this time period (2.3 and 1.8 per 100,000). Incidence increased steeply with age, being 0.25 per 100,000 below the age of 45 years, 3.4 among the 45–70 years age group, and 5.9 after age 70+ years.

Conclusions: This is the first meta-analysis of ALS epidemiological data and represents the largest reference population presented to date.

C66 ANGIOGENIN POLYMORPHISMS MODIFY AGE AT ONSET IN SPORADIC ALS

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Background: Variations in angiogenin (ANG), a hypoxia-inducible gene, have been implicated as risk factors for motor neuron degeneration (1).

Objectives: To investigate the association and modifier effects of ANG gene polymorphisms with sporadic ALS (SALS) in a family-based and case-control cohort.

Methods: We investigated the association of ANG gene polymorphisms in a large North American Caucasian family-based and case-control cohort (n=1987). Genotyping was performed using TaqMan® SNP Genotyping Assays. Data were analysed using SPSS, FBAT and Haploview. We genotyped three SNPs (rs1010461, hCV2742351, rs11701) spanning the ANG

gene including the synonymous polymorphism which was earlier found to be associated with ALS in the Irish and Scottish populations (1).

Results: The T allele of rs11701 was associated with SALS in the trio group ($p=0.0027$) and the pedigree model ($p=0.033$). There was no association in the discordant sib-pair or case-control models. Haplotype analysis of the three SNPs in the trio model revealed that the A-C-G haplotype was protective ($p=0.0058$) whereas the A-C-T haplotype was associated with increased risk of disease ($p=0.045$).

The GG genotype of rs11701 advanced the age at onset of SALS by 14.2 years compared with the GT and TT genotypes on Kaplan-Meier analysis (Log-rank=4.96, $p=0.026$). In contrast, the AA genotype of rs1010461 was associated with an earlier age at onset of disease (Log-rank=4.12, $p=0.042$).

Conclusions: Our findings are contrary to those reported earlier and implicate the T allele of rs11701 as the detrimental allele for SALS in North America. The evidence suggests that ANG is a modifier gene for SALS and plays a significant role in affecting the age at onset of disease.

Acknowledgements: We thank the patients with ALS and their families for their participation in this study. Without their help this work would not have been possible. This research was supported by NINDS (NS050641, NS046535), Les Turner ALS Foundation, V.E. Schaff ALS Research Fund, H. Post Research Professorship, Wenske Foundation, Falk Medical Research Trust, Les Turner ALS Foundation / Herbert C. Wenske Foundation Professorship and The David C. Asselin MD Memorial Fund.

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C67 EVALUATION OF LINKAGE OF ALS/FTD TO CHROMOSOME 9Q WITH THE ESTABLISHMENT OF NEW LINKAGE TO 9P21

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Background: ALS, a clinically and genetically heterogeneous disease with several genetic loci identified, is sometimes associated with frontotemporal dementia (FTD). FTD constitutes a degenerative disorder of the frontal and temporal lobes with onset of symptoms between the ages of 35 and 75 years and accounts for 12.5–16.6% of all primary degenerative dementias. FTD is now well recognized in ALS, and ALS could be associated with dementia with an incidence varying from 3% to 15%. Previous reports of linkage of ALS/FTD to 9q21 have not been verified by subsequent studies.

Objectives: To ascertain the linkage of ALS/FTD.

Methods: Fifteen ALS/FTD families were included in an affected relative pair analysis of 43 SOD1 negative ALS and ALS/FTD families which were screened with 405 fluorescent-labelled MapPairs® human DNA markers covering the entire genome at 20 cM. Additional microsatellite markers were selected for dense mapping under peaks with lod scores >1. Genotyping and sequencing were both performed on Beckman Coulter CEQ 8000 Genetic Analysis Systems. Two-point and two-point heterogeneity LOD score (HLOD) analyses were computed using FASTLINK and multipoint analyses were analysed by SIMWALK.

Results: There were 80 affected individuals, of which 49 subjects were genotyped. Three families with positive scores on 9q21 were excluded from that locus. No mutations were detected in the *Tau* gene or the VCP and 92 genes. The ARP screen suggested linkage to 9p. Detailed genotyping on chromosome 9 showed a location score of 5.36 at 51.53 cM, and a Hetlod of 9.9 at 51.06 cM by Simwalk. The multipoint LOD score and multipoint heterogeneity score returned by Fastlink overlapped with each other at 47.57 cM with identical value of 7.74. The maximum two-point LOD score returned by Fastlink was 6.8 at D9S259 for the fifteen ALS/FTD families. Based on LOD scores and haplotype analysis 9 out of 15 ALS/FTD families were linked to 9p21. F638, F9969 and F9748 were all possibly linked to this new locus. A centromeric crossover between D9S1678 and D9S259 was found in F8462 and F9029 and a telemetric crossover between D9S1846 and D9S1684 was present in F8462, F7810 and F638, which defines the MCR of the new locus being 12.49 cM on 9p21 flanked by D9S1684 and D9S1678. Two brains were available for pathological evaluation, and they showed remarkable circumscribed frontal and temporal atrophy, neuronal loss and gliosis and ubiquitin skein-like and Lewy-like inclusions in anterior horn neurons and cytoplasmic neuronal inclusions in hippocampal dentate gyrus.

Discussion and conclusions: The linkage of ALS/FTD to 9q was not validated and robust evidence for a major novel locus on 9P21 for ALS/FTD was obtained. Efforts are underway to identify the gene defect.

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C68 COMPREHENSIVE ANALYSIS OF CAUSATIVE AND RELATED GENES FOR ALS USING A HIGH THROUGHPUT DNA MICROARRAY-BASED RESEQUENCING SYSTEM

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Background: The list of causative genes for familial ALS (FALS) has been expanding in accordance with the recent progress in molecular genetics. Alterations of these genes may be correlated with the pathogenesis of sporadic ALS due to reduced penetrance of the mutations. Furthermore, some alterations may contribute to ALS as disease susceptibility genes. Under this situation, comprehensive analysis of causative and related genes should be of great importance not only for molecular diagnosis, but also for elucidating molecular epidemiology and dissecting molecular pathogenesis of ALS. With this background, we have constructed a high throughput gene analysis system and conducted comprehensive mutational analysis of ALS-related genes.

Objectives: To develop a DNA microarray-based high throughput resequencing system and to conduct comprehensive analysis of causative and related genes for familial and sporadic ALS patients.

Methods: We have developed a DNA microarray-based high throughput resequencing system employing GeneChip Custom Resequencing System (Affymetrix). The microarray includes all the exon and flanking intron sequences of three causative genes (SOD1, ALS2 and DCTN1) and seven related genes (SLC1A2, SMN1, LIF, RNF19, ADAR2, CNTF and VEGF) for ALS. Forty-two ALS patients, including seven familial and 35 sporadic cases were analysed. Genomic DNA samples were amplified using specific PCR primers, and processed according to the manufacturer's instructions. The handling of numerous PCR samples was accomplished by employing a robotics system to further enhance the throughput.

Results: We have completed resequencing of approximately 1 Mb (25 kb each for 43 patients), and identified five causative mutations. Two SOD1 mutations including one novel mutation and one previously identified mutation were found in seven familial cases. The patient with the novel mutation was characterized as late-onset, slowly progressive ALS. Three mutations were identified in 35 apparently sporadic patients, which include one novel DCTN1 mutation and one previously known SOD1 mutation, and one SMN1 homozygous exon7 deletion mutation. Both the patient with the DCTN1 mutation and the patient with the SOD1 mutation presented typical ALS features with predominantly lower motor neuron involvement, clinically indistinguishable from other ALS patients. In addition, 27 single nucleotide substitutions were identified in the 10 genes. Among them, 11 substitutions (40%) were novel, including five non-synonymous heterozygous substitutions in ALS2 in six sporadic ALS patients and one non-synonymous single nucleotide substitution in VEGF in one atypical ALS patient.

Conclusions: Our approach for comprehensive analysis of causative and related genes has been proved to be fruitful for familial and sporadic ALS in identifying causative mutations and novel variations potentially associated with disease risks. The DNA microarray-based resequencing system is highly useful for this approach, contributing not only to molecular diagnosis but also to molecular epidemiology and molecular dissection of ALS.

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SESSION 9A CELLULAR TRANSPORT AND TRAFFICKING

C69 CHARACTERIZATION OF AMYOTROPHIC LATERAL SCLEROSIS-LINKED PRO56SER MUTATION OF VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN B (VAPB/ALS8)

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Background: The Pro56Ser (P56S) mutation in vesicle-associated membrane protein-associated protein B (VAPB) is the most recently identified ALS-linked gene (ALS8) and causes autosomal-dominant motoneuronal diseases (MND). VAPB is a homolog of aplysia VAP33, involved in secretion of neurotransmitter and known to localize in ER-Golgi apparatus, but its physiological role remains unclear. It was reported that the P56S mutation induces a shift of VAPB from endoplasmic reticulum (ER) to non-ER compartments; it still remains unclear how the P56S mutation causes MND.

Objectives: To characterize the properties of P56S-VAPB that may be related to the onset of ALS.

Methods: To clarify how the P56S mutation affects the properties of the VAPB protein, we first investigated the interaction of P56S-VAPB with its binding proteins by pull-down analyses. Next, we compared solubility of wt-VAPB and P56S-VAPB in a 1% Triton-X100 (Triton)-containing buffer. For these purposes, wt-VAPB or P56S-VAPB, transiently overexpressed in motoneuronal NSC34 cells, were fractionated into Triton-soluble or -insoluble fractions and subject to immunoblot analysis. We further investigated the abnormal localization of P56S-VAPB by immunocytochemistry and sucrose density gradient centrifugation. For investigation of VAPB function, we used the unfolded protein response (UPR) assay because VAP and its yeast homolog SCS2 are suspected to be involved in UPR.

Results: Although the P56S mutation did not affect the homodimerization with VAPB or the heterodimerization between VAPB and VAPA or synaptobrevin (vesicle associated membrane protein-1 and -2), it drastically decreased the solubility of VAPB protein and caused a shift in the localization of VAPB from ER to non-ER compartment. Furthermore, P56S-VAPB enhanced insolubility of co-expressed wt-VAPB, but not VAPA, VAMP1 or VAMP2. Enforced expression of wt-VAPB triggered UPR, but P56S-VAPB did not, indicating that P56S-VAPB is a non-functional mutant. Any tested P56X-VAPB point-mutant (X=A, K, and D) or VAPB with a deletion 56th proline showed the same characteristic as P56S-VAPB, suggesting that the proline at the 56th position plays an important role in the proper structure of VAPB and its physiological functions.

Discussion and conclusions: We demonstrate that P56S-VAPB is more insoluble in the Triton-containing buffer than wt-VAPB, indicating that it is misfolded. Accordingly, P56S-VAPB loses the function to induce UPR, an ER reaction to ER stress that is triggered by overexpression of wt-VAPB. P56S-VAPB interferes with the folding of co-expressed wt-VAPB possibly because it still retains the ability to be firmly dimerized with wt-VAPB despite its misfolding and mislocalization. As a result, co-expression of P56S-VAPB enhances the insolubility of wt-VAPB and prevents wt-VAPB from triggering UPR. We conclude that P56S-VAPB is a loss-of-function mutant. We further conclude that it dominant-negatively affects the function and localization of wt-VAPB. These unique characteristics of P56S-VAPB may play an important role in the pathomechanism underlying motoneuronal degeneration linked to ALS8.

C70 ALS2 IS LOCALIZED TO ENDOSOMES IN PRIMARY CULTURED HIPPOCAMPAL NEURONS AND IMPLICATED IN AXON ELONGATION

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Background: The *ALS2* gene was identified as a causative gene for a number of juvenile recessive motor neuron diseases (MNDs), such as ALS2, PLSJ, HSP and IAHSP. It has been postulated that a functional loss of the ALS2 protein (ALS2/alsin) leads to selective motor neuron degeneration and MND. Recently, we have demonstrated that ALS2 acts as a guanine nucleotide exchange factor for small GTPase Rab5 and enhances early endosome fusion through the activation of Rab5. Thus, ALS2 may play an important role in the maintenance of motor neurons via regulating membrane trafficking. However, detailed distribution and functions of ALS2 in neurons are still unclear.

Objectives: The purpose of this study is to identify subcellular localization of neuronal ALS2 at different stages of neural development using primary cultured hippocampal neurons as a model for developing neurons. Further, we also examined whether levels of ALS2 expression affect axon outgrowth by comparing the length of axon in the *ALS2* deficient, ALS2 overexpressing, and wild type neurons.

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Methods: Hippocampal neurons were isolated from hippocampi dissected from post- neonatal day 1 mouse, and cultured for different periods of time to examine the ALS2 localization in the cells at different developmental stages. ALS2 was immunocytochemically detected and analysed by the Leica TCS-NT system. Further, to measure the length of axon in stage 3 neurons, 2×10^4 cells were plated onto poly-D-lysine coated round glasses. After 36 h, the cells were fixed, permeabilized, and stained with anti-MAP2 antibody and Alexa594-Phalloidin. The longest neurite with attenuated MAP2 staining was defined as the axon. Captured cell images were analysed by ImageJ and axon length was determined.

Results: In stage 3 neurons, ALS2 was distributed to cytoplasm and patchy membrane structures. ALS2 was also enriched in membrane ruffles at the growth cone colocalizing with F-actin. In stage 5 neurons, ALS2 was localized to endosomes both in dendrites and in the axon, which were partially labelled with Alexa-594 transferrin. The ALS2-positive axonal endosomes were also colocalized with synaptophysin prior to the synapse formation. Notably, at stage 3, although no significant differences in the outgrowth of MAP2-positive dendrites among different genotypes were observed, ALS2 deficient neurons extended shorter axons than wild-type ones, while overexpression of ALS2 promoted axon growth.

Discussion and conclusions: These observations suggest that ALS2 is implicated in axonal development in immature neurons. A unique endosomal localization of ALS2 in matured neurons implies that ALS2 might regulate membrane trafficking through the endosome fusion, thereby mediating the survival and maintenance of motor neurons. Further studies on the neuronal functions of ALS2 will lead to better understanding of the pathogenesis for ALS2-linked, as well as other, MNDs.

C71 ALS2 IS A NOVEL RAC1-REGULATED MACROPINOSOMAL RAB5GEF THAT MEDIATES INTERCONNECTION BETWEEN DISTINCT ENDOCYTIC PATHWAYS

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Background: ALS2/alsin is a causative gene product for several juvenile recessive motor neuron diseases (MNDs). ALS2 acts as a guanine nucleotide exchange factor (GEF) for Rab5 (Rab5GEF) and is implicated in endosome fusion. It has been postulated that a perturbation of endosome dynamics caused by loss of the ALS2 function might underlie motor dysfunction. ALS2 is preferentially localized onto endosomal vesicles in cultured neurons, while it is sequestered in cytoplasm in most non-neuronal cells. Therefore, intracellular distribution of ALS2 might be regulated by the upstream signal(s), thereby modulating endosome dynamics. However, the molecular mechanisms by which ALS2 is activated/redistributed in cells are still unknown.

Objectives: The purpose of this study is to identify the upstream activator(s) for ALS2. We also investigated cellular processes that ALS2 regulates. This study will lead to a better understanding of the pathogenesis underlying the ALS2-linked MNDs.

Methods: We performed *in vitro* binding and co-immunoprecipitation analyses to identify the small G protein that binds to ALS2. Subsequently, to investigate the physiological significance of the identified interaction, we conducted co-transfection experiments of ALS2 with its interactor and/or activator in HeLa cells. The ALS2 localization and the ALS2-positive endocytic vesicles were characterized using immunocytochemical methods. We also conducted uptake analyses of fluorescently-labeled molecules, such as dextran and transferrin, in cultured cells, to identify the ALS2-regulated endocytic pathways.

Results: Here we showed that ALS2 preferentially interacted with an activated form of Rac1 as a novel Rac1 effector. Interestingly, cytoplasmic ALS2 was recruited to membrane ruffles and then relocalized onto nascent macropinosomes via macropinocytosis, a mode of endocytosis, upon Rac1 signaling. Thus, Rac1 acts as an upstream activator for ALS2. ALS2 is the first Rab5GEF that is positively regulated by a Rho member, Rac1. At later endocytic stages, macropinosomal ALS2 regulated fusion between the ALS2-localized macropinosomes and the CME-derived endosomes, depending on the ALS2-associated Rab5GEF activity. These findings reveal a fundamental role of ALS2 being implicated in the spatiotemporal regulation of Rac1-Rab5 signaling.

Discussion and conclusions: We here demonstrate that the Rab5GEF ALS2/alsin mediates the interconnection between Rac1-induced macropinosomes and CME-derived endosomes. Endocytosis is crucial to numerous cellular processes and involves multiple internalization mechanisms, such as macropinocytosis, CME, and caveolin-dependent endocytosis. Thus, we propose that both clathrin-dependent and independent endocytic mechanisms that are coordinately interconnected by ALS2 are crucial for the survival of motor neurons. Future studies on the ALS2 functions in neurons will provide further insights into the pathogenesis for MNDs caused by the ALS2 mutations.

C72 DEREGULATION OF PKN BY GLUTAMATE AND ALS-LINKED MUTANT SOD1 INDUCES CHANGES IN NEUROFILAMENT ORGANIZATION AND TRANSPORT

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Background and objectives: Protein kinase N (also known as Protein kinase C-related kinase-1; PRK1) is a serine/threonine kinase with a C-terminal catalytic domain similar to that found in PKC and a unique N-terminal regulatory region. Within neurons, it phosphorylates both neurofilaments and the microtubule-associated protein tau, and this has led to the suggestion that abnormal PKN activity contributes to the pathogenesis of some neurodegenerative diseases. Indeed, PKN can be cleaved by caspase family proteases to release a deregulated C-terminal fragment containing the catalytic domain devoid of N-terminal regulatory moieties. Such a cleaved deregulated PKN fragment is generated in a number of experimental models of neurodegeneration. Here, we have investigated the role of PKN in ALS.

Method and results: We initially investigated the effect of glutamate on PKN activity. Treatment of cultured rat cortical neurons with 100 μM glutamate for up to 30 min led to a significant increase in PKN activity, as determined by *in vitro* kinase assays. This activation was shown to be mediated via NMDA-type receptors. Extended treatment of neurons with glutamate induced proteolytic cleavage of PKN to release a predicted deregulated C-terminal fragment. This cleavage was blocked by the caspase inhibitor ZVAD at 100 μM.

We next investigated whether cleavage of PKN to produce such a deregulated fragment also occurs in G93ASOD1 transgenic mice. Immunoblots of brain and spinal cord samples from G93ASOD1 and control mice revealed the presence of the cleaved PKN fragment specifically in spinal cords of G93ASOD1 mice. Additionally, immunohistochemical staining revealed that PKN was present in neurofilament accumulation containing motor neuron cell bodies of these mice.

To determine which region of neurofilament light chain (NFL) is phosphorylated by PKN, we performed *in vitro* kinase assays with different domains of NFL. PKN phosphorylated the head domain of NFL; such phosphorylation is believed to regulate the assembly properties of neurofilaments. We therefore examined neurofilament assembly and detergent solubility in SW13 cells' cortical neurons following modulation of PKN activity. The cleaved deregulated PKN induced subtle changes to neurofilament architecture. Finally, we studied whether PKN influenced axonal transport of neurofilaments in cultured neurons. Transfection of wild-type PKN had no effect on neurofilament transport but transfection of the cleaved deregulated PKN induced a marked decrease in neurofilament transport.

Discussion and conclusions: We have shown that two insults associated with ALS, glutamate excitotoxicity and mutant SOD1, both induce changes in PKN activity. PKN phosphorylates the head domain of NFL and a deregulated PKN fragment induces changes in neurofilament assembly properties and axonal transport. Deregulation of PKN may thus be part of the pathogenic process in ALS. This work was supported by grants from the MND Association and Medical Research Council.

C73 REVERSIBLE DISRUPTION OF RETROGRADE AXONAL TRANSPORT 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. As suggested in other polyglutamine diseases, pathogenic AR-mediated transcriptional dysregulation has been implicated in the pathogenesis of SBMA. Mouse models of SBMA suggest that dysfunction of motor neurons precedes neuronal cell death in the process of neurodegeneration.

Objectives: The aim of this study is to elucidate the mechanism by which motor neuron function deteriorates prior to neuronal cell death in SBMA.

Methods: We used a transgenic mouse model of SBMA carrying the full-length human *AR* gene harboring 24 (AR-24Q) or 97 CAGs (AR-97Q). Immunohistochemistry and immunofluorescent analysis were performed on mouse tissues. Axonal transport in the mice was investigated using retrograde neurotracer labelling and nerve ligation. The expression levels of axon motor proteins were determined using in situ hybridization and quantitative RT-PCR.

Results: AR-97Q mice demonstrated a striking accumulation of both phosphorylated and non-phosphorylated NF-H in the skeletal muscle, whereas this finding was not observed in AR-24Q or wild-types. Similar accumulation was also observed for middle molecular weight NF (NF-M) and synaptophysin. Anti-NF immunostaining demonstrated that intramuscular NF accumulation was detectable from as early as seven weeks old, prior to the onset of muscle weakness in this mouse model, and aggravated thereafter. To elucidate the molecular basis for abnormal distribution of NF, we studied axonal transport in this mouse model of SBMA. Retrograde neurotracer labeling and nerve ligation demonstrate that the retrograde

transport is disrupted prior to the onset of neurological symptoms. To elucidate the molecular mechanism compromising retrograde axonal transport, we examined the expression level of axon motors and associated proteins. The spinal motor neurons demonstrated a lowered protein level of the largest subunit of dynactin (dynactin1) in spinal cord sections from AR-97Q mice. In the ventral root, decrease in dynactin1 levels was significant before the onset of motor symptoms. Although the protein level of dynein heavy chain was slightly diminished in the advanced stage, this phenomenon was not observed before the onset of symptoms. In situ hybridization and quantitative RT-PCR demonstrated that the mRNA level of dynactin1 was markedly repressed in the spinal cord of AR-97Q mice in the pre-onset stage.

Discussion and conclusions: The present study indicates that the accumulation of axonal components in distal motor axons appears to be a substantial pathology associated with degeneration of lower motor neurons. Although previous studies have suggested a direct inhibition of axonal transport by mutant AR protein within motor axons, our results demonstrate that polyglutamine-mediated transcriptional dysregulation of dynactin1, the p150 subunit of dynactin, in affected neurons is a basis for perturbation of retrograde axonal transport in the SBMA mouse.

C74 SOD1 AGGREGATES GENERATED WITHIN MOTONEURONAL DENDRITES/ CELL BODIES MOVE INTO AXONS BEFORE DISEASE ONSET IN A G93ASOD1 TRANSGENIC MOUSE MODEL

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Background and objectives: Aggregation of mutant SOD1 proteins is suggested to be responsible for the selective loss of motor neurons in SOD1-related ALS, although the mechanisms underlying such aggregates' related toxicity have been elusive. Since the subcellular localization of the aggregates will provide an important clue in understanding the toxicity, we investigated the location of the SOD1 aggregates within motor neurons and how the localization changed during disease progression in a $SOD1^{G93A}$-Tg mouse model.

Methods: We analysed the localization of SOD1 aggregates by two different methods. First we performed subcellular fractionation of spinal cords producing five fractions in which typical organelles, as well as fragmented axons, were independently enriched. Secondly, we carried out an area-specific isolation utilized laser-assisted microdissection technique. From the frozen sections of non-fixed spinal cords, we isolated several areas enriched with cell bodies of motor neurons, dendrites of motor neurons/neighbouring glial cells, etc. These fractions were subjected to SDS-PAGE and Western blot analysis, and the properties of SOD1 aggregates were examined.

Results: The subcellular fractionation analysis revealed that SOD1 aggregates were first detected in the fractions enriched with mitochondria and axons a long time before disease onset, and then increasingly accumulated into the latter fraction by disease onset. With disease progression, the aggregates rapidly spread into other fractions, other than the fraction composed of cytosolic soluble proteins. The area-specific isolation technique revealed that SOD1 aggregates were first detected within the areas including cell bodies and dendrites of motor neurons, followed by their detection within the area containing motoneuronal axons. Combined with these and other data, SOD1 aggregates are thought to be generated within dendrites/cell bodies and then move into the axons within motor neurons before disease onset.

Discussions and conclusions: We previously found that calcium-permeable AMPA-type glutamate receptors promote generation of SOD1 aggregates with an increased cellular oxidative stress in $SOD1^{G93A}$-Tg mice (1). AMPA receptors are localized to the dendrites of motor neurons and largely contribute to the excitotoxicity that generates oxidative stress. Since oxidative stress promotes the conversion of mutant SOD1 into aggregates, our findings imply a direct connection between excitotoxicity and generation of SOD1 aggregates within dendrites. Considering the remarkable accumulation of SOD1 aggregates within axon-enriched fraction/area by disease onset, the toxicity of aggregates may be related to the translocation of the aggregates from dendrites/cell bodies to axons.

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SESSION 9B DATABASES

C75 HARMONIzATION OF DATABASES

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No abstract available.

C76 ALS PATIENT CARE DATABASE IN JAPAN

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Background: The ALS Patient Care Database was created to improve the quality of care for patients with ALS in the United States, and it has provided valuable data on physicians' practices and patient-focused outcomes (1).

Objectives: We started an ALS database in Japan to describe the status and trends in patients' QOL, clinical conditions, therapeutic interventions, etc.; to evaluate the effects of ALS on caregivers; and to help researchers with regard to instruments and items useful for observational and interventional studies.

Methods: The database was open to all neurologists in Japan. Data were collected at intervals of six months. Patient-reported data included demographic information, initial diagnosis, duration of symptoms, interventions, self-reported health care, satisfaction with medical care, ALSAQ-40, etc. Physician-reported data included date of diagnosis, diagnostic criteria, region affected by ALS, ALSFRS-R, WFN criteria, etc. Caregiver-reported data included demographic information, relationship to patient, employment status, general health status, SF-36, carer status, source of payments for caregiving, etc. Data-collection forms were submitted to a single data center.

Results: From October 2001 to November 2004, data on 89 patients at about 40 clinical sites were collected. Patients had a mean age of 61.1 (SD 11.2) years and 63% were men. Almost all patients (96%) had sporadic ALS, and 45% had classical ALS. The mean interval from onset of the disease was 37.7 months (SD 30.1, range 1–122). About half of the patients were satisfied with the quality of their care. The mean age of caregivers was 54.0 (SD 13.9) years and 76% of them were female. The primary caregiver was the spouse (70%). About 70% of the caregivers were paid for their service by public money. One-quarter of them had stopped other paid employment. The QOL data (SF-36) documented that caregivers had stress and emotional burdens attributed to the disease and to caregiving. The data indicate that the Japanese version of the ALSAQ-40 (2) had high validity and was likely to be useful in evaluating the QOL of ALS patients in Japan. Physicians (using the ALSFRS-R) and caregivers were consistent in their evaluations of patients' abilities to carry out daily activities.

Discussion and conclusions: Longitudinal data will be needed to reveal trends in clinical conditions and interventions, and to examine the relationship between them. This database appears to provide valuable information in ALS patient care. All neurologists practising in Japan are welcome to contribute to the database and to use the data.

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C77 TREATMENT OF PATIENTS WITH ALS IN THE COMMUNITY: NEW INSIGHTS FROM THE WEB-BASED ALS PATIENT CARE DATABASE

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Background: Data on outcomes for patients cared for in arge ALS centers comes from the ALS C.A.R.E database. New data were gathered from patients who receive care in the community via a web-based ALS registry.

Objective: To compare outcomes of patients treated in the community with those treated in large specialized ALS clinics.

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Methods: Treatment and outcomes were compared from the ALS C.A.R.E. database with data entered in the web-based, patient-driven database, ALS Connection, supported by a grant from the MDA. The website was launched in January 2006. This analysis compares the first 111 patients enrolled in the ALS Connection (Web) with more than 6000 patients enrolled in the ALS C.A.R.E. database (CARE), 90% of whom are cared for in large ALS clinics.

Results: More Web patients obtained their information regarding ALS from the internet (CARE 3% vs. Web 28%). In general the impact of ALS was greater on Web patients: fewer Web patients could perform more than 50% of daily activities themselves (CARE 54% vs. Web 42%); more Web patients accomplished much less due to the disease (CARE 25% vs. Web 65%). However, more patients were satisfied with their care in the ALS CARE database (CARE 91% vs. Web77%).

When given the diagnosis of ALS, 71% of CARE patients received adequate information about patient advocacy organizations compared with 47% of Web. More Web patients were dissatisfied with receiving the diagnosis: too little information (CARE 2% vs. Web 17%); no hope given (CARE 3% vs. Web 13%); insensitive delivery (CARE 2% vs. Web 46%).

Access to symptomatic therapy for drooling was only 3% in the Web vs. 32% in the CARE group. Similarly, fewer Web patients used helpful therapies for pseudobulbar affect: amitriptyline (CARE 24% vs. Web 5%); SSRI (CARE 47% vs. Web 7%); other (e.g. dextromethorphan)(CARE 61% vs. Web 8%).

Web patients used less therapy: occupational therapy (CARE 59% vs. Web 42%); speech therapy (CARE 61% vs. Web 24%); nutrition (CARE 66% vs. Web 35%); social worker (CARE 65% vs. Web 27%); psychologist (CARE 65% vs. Web 14%); home nurse (CARE 63% vs. Web 27%). Recommendation for enteral feeding was similar in both cohorts but utilization was less in CARE (CARE 30% vs. Web 42%). Utilization of non-invasive ventilation was similar in Web and CARE patients.

Conclusions: Patients with ALS who enrolled in a web-based registry were more impacted by ALS, were generally less satisfied with their care, had a more unsatisfactory experience in learning about the diagnosis, and less access to symptomatic therapy than those in the ALS C.A.R.E. database. ALS patient registries are needed to identify disease impact, patient needs and treatment utilization. Patients from ALS clinics and patients who access a web registry appear to have different experiences. Further research is needed to identify the reasons for these differences and how stable these differences are.

C78 CHALLENGES OF DATA MANAGEMENT IN WEB-BASED MULTI-SITE CLINICAL TRIALS IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: The Neurology Clinical Trials Unit (NCTU) of the Massachusetts General Hospital serves as the coordination center for several multi-site clinical trials in amyotrophic lateral sclerosis (ALS). Efficient data management (DM) is one of the important aspects of successful conduct of clinical trials.

Objectives: Creating an efficient and compliant environment for DM of multi-site clinical trials in ALS is one of the major objectives for the clinical trials coordination center. Well-organized and competent data management will reduce time in data cleaning and will allow faster locking of the database for biostatistical analyses.

Methods: NCTU has conducted multi-site clinical trials in ALS for more than five years and has accumulated sufficient empirical data, and developed methods to maximize efficiency of DM in clinical trials. These methods may be divided into two areas, general DM and ALS-specific DM.

The following factors may determine a successful clinical trial from the DM perspective:

- Involvement of DM at the start of the trial into protocol design, Case Report Forms (CRFs) and EDC system specifications development
- Constant communication with site users and feedback solicitation
- Initiation of user training as combination of in-class and web-based sessions and periodical brief refreshment classes
- Unambiguous designation of tasks for data management and study monitors in data cleaning and query closing

Clinical trials in subjects with ALS have certain ALS-specific factors that must be discussed and resolved prior to the commencement of the trial:

- Clear definition of outcome measures
- Development of standard ALS-specific common data elements (CDE) that guarantee consistency and reliability of trial data. These data elements include CRF design, associated with the form data dictionary, logic checks, data ranges, help files and reports.
- Introduction of new types of visits (site, home, phone) for 'survival' trials
- Education of sponsors, study and medical monitors on ALS-specific adverse events reporting

Results: NCTU has developed certain approaches, techniques and recommendations on DM of clinical trials in subjects with ALS. These approaches were successfully used in several multi-site trials. Future standardization of

ALS-specific CDE and more effective utilization of accumulated know-how are recommended.

Conclusions: The following are recommended to the ALS research community to successfully implement this task:

- Implement efficient data management
- Develop standards for ALS-specific CDE
- Clearly define outcome measures
- Use web portal and conferencing for communications with site users
- Develop multi-layer continuous data capture training program
- Define clear line of responsibilities between DM and study monitors
- Define data exchange mechanism with central laboratory
- Allocate sufficient time for testing and validation of EDC system

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SESSION 10A ROLE OF NON-NEURONAL CELLS

C79 EMERGING ROLE FOR ASTROCYTES IN THE PATHOGENESIS OF ALS

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The pathogenesis of ALS is likely to involve disturbed intercellular communications between motor neurons and other cell types. Fibroblast growth factor-1 (FGF) can contribute to induce the sustained astrocyte activation characteristic of ALS. FGF-1 is highly expressed in motor neurons. It can be released from cells during oxidative stress, which might occur from SOD-1 aberrant function. FGF-1 potently stimulates NGF expression and release in astrocytes. NGF in turn induces apoptosis in motor neurons expressing the p75 neurotrophin receptor (p75NTR) by a mechanism involving nitric oxide (NO) and peroxynitrite formation. In addition, FGF-1 increased the expression of inducible nitric oxide synthase and NO production in astrocytes, making adjacent motor neurons vulnerable to NGF-induced apoptosis. Since p75NTR is re-expressed only by affected motor neurons in ALS, it is proposed that astrocytic NGF or high molecular weight NGF species, may serve to eliminate damaged neurons. Spinal cord astrocytes isolated from transgenic SOD1G93A rats displayed increased NO production and spontaneously induced apoptosis of co-cultured motor neurons.

FGF-1 also activates the redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) in astrocytes. Accordingly, both Nrf2 and Heme oxygenase-1 levels were increased and colocalized with reactive astrocytes in the degenerating lumbar spinal cord of rats expressing the ALS-linked SOD1 G93A mutation. Because Nrf2 increases glutathione (GSH) biosynthesis, we investigated the role of GSH production by astrocytes on p75NTR-dependent motor neuron apoptosis. The combined treatment of astrocytes with FGF-1 and t-butylhydroquinone (tBHQ) increased GSH production and secretion, preventing motor neuron apoptosis. Activation or overexpression of Nrf2 in astrocytes expressing the SOD-1 mutations completely prevented motor neuron apoptosis in co-cultures and prevented p75-dependent apoptosis. We hypothesizes that SOD-1 mutations linked to ALS sensitize astrocytes to FGF-induced oxidative damage, leading to a decrease in the neurotrophic and antioxidant support for motor neurons.

C80 GLUTAMATE EXCITOTOXICITY IN ALS: THE SEARCH FOR PROTEIN INTERACTORS OF EAAT2

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Background: Many theories for the pathogenesis of amyotrophic lateral sclerosis (ALS) have been proposed including that of glutamate mediated excitotoxicity. Glutamate is the major excitatory neurotransmitter in the mammalian CNS and in excess can be toxic to neuronal cells. Glutamate levels are tightly controlled by glutamate transport proteins, the main functional transporter being the astrocytic glutamate transporter EAAT2. Evidence for glutamate excitotoxicity in ALS comes from reports of raised levels of glutamate in the cerebrospinal fluid and the finding of reduced levels of EAAT2 in the CNS of ALS patients. The cause of reduced astrocytic expression of EAAT2 in ALS patients is unknown. Investigations into the mRNA expression and genomic sequence of EAAT2 have found no differences between patients and controls. An alternative theory is that proteins interacting with EAAT2 are abnormal in ALS and it is the abnormalities in these proteins that lead to impaired EAAT2 function and glutamate excitotoxicity in the disease. No proteins that interact with EAAT2 have as yet been reported in humans, while only a single protein, Ajuba, a group 3 LIM protein, has been reported to interact with glt-1, the homologue of EAAT2, in rats. We are investigating this interaction in humans and have undertaken a yeast two hybrid screen to identify additional interacting proteins.

Objectives: To identify proteins that interact with EAAT2 and confirm the EAAT2/Ajuba interaction in human cells. To investigate whether alterations in any of these interacting proteins are responsible for the reduced expression or altered function of EAAT2 in ALS.

Methods: We used a yeast two-hybrid system to identify proteins that interact with EAAT2. A C-terminal, predicted cytoplasmic fragment of EAAT2 was used as bait to screen a pretransformed adult human brain cDNA library (Clontech). The full coding sequences of the strongest potential positives from the yeast screen, Ajuba and EAAT2 were cloned into mammalian expression vectors with epitope tags. Transfected cells were visualized using immunocytochemistry and the tagged proteins extracted and used in co-immunoprecipitation experiments.

Results: We have identified 54 proteins that interact with our EAAT2 bait in a yeast two hybrid system.

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Co-immunoprecipitation and colocalization experiments to date show that three of the potential positives and Ajuba each colocalise and interact with EAAT2 in mammalian cells.

Discussion and conclusions: We have discovered three proteins that interact with EAAT2 and confirmed the EAAT2/Ajuba interaction in mammalian cells. The next challenge is to investigate if and how these interactions might influence EAAT2 function and elucidate whether changes in any of these proteins have relevance in ALS pathogenesis.

C81 ONSET AND PROGRESSION IN INHERITED ALS DETERMINED BY MOTOR NEURONS AND MICROGLIA

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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 not known.

Objectives: To determine the cell types in which mutant SOD1 acts to generate toxicity to the motor neurons in ALS.

Methods: We have generated a mouse ubiquitously expressing a 'floxed' mutant $SOD1^{G37R}$ transgene ($LoxSOD1^{G37R}$) which can be removed within specific cell populations by the action of Cre recombinase. To delete mutant SOD1 within motor neurons or microglia/macrophages, $LoxSOD1^{G37R}$ mice were mated with Islet1-Cre or CD11b-Cre mice, respectively. The disease onset, duration, and survival of these cohorts were monitored.

Results: Removing mutant SOD1 from motor neurons extends the age of disease onset, and slows the early phase of disease progression, indicating mutant action in neurons as an initiating factor in triggering disease. More importantly, silencing of SOD1 mutant expression selectively within microglial cells and peripheral macrophages has minimal effect on age of disease onset, but dramatically slows disease progression (extending survival after onset by 75 days).

Discussion and conclusions: Microglia and/or macrophages play a central role as a determinant of the timing of disease progression in ALS. Mutant toxicity within microglia and/or macrophages leads to the non-cell autonomous killing of motor neurons, following the disease initiation caused by the toxicity within motor neurons. Contributions of other non-neuronal cell types, including astrocytes and muscles are being tested.

C82 GENE EXPRESSION CHANGES IN MICROGLIAL CELLS CARRYING THE HUMAN MUTANT SOD1 GENE

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Background: Microglia are often referred to as the macrophages of the brain. They play an active role in surveillance of CNS tissue homeostasis and respond to perturbations with acquisition of a more activated phenotype. Reactive microglia are seen in inflammatory, infective and neurodegenerative conditions. They are present in affected tissues in human cases of ALS and in mutant SOD1 transgenic mice. The hypothesis of non-cell autonomous damage to motor neurons in ALS proposes that altered properties of glial cells, carrying the mutant SOD1 gene, could be responsible for the toxicity towards or failure to support the neighbouring motor neurons.

Objectives: Our objective is to investigate the effects of mutant SOD1 on the microglial transcriptome by comparing the mRNA profile of non-transgenic and mutant SOD1 G93A transgenic microglial cells isolated from animal models of the disease.

Methods: We established a protocol to obtain highly purified microglial cultures from non-transgenic and human SOD1 G93A transgenic neonatal mice. The purity of cultures was determined by immunocytochemistry using a macrophage specific marker F4/80. The mRNA of the purified microglial cells was used in rounds of amplification, in vitro transcription, labelling, fragmentation and hybridized onto the mouse genome 430 2.0 microarray using Affymetrix technology. Pathway Architect software was used to pull out the key affected pathways in the transgenic microglial cells. Differences in gene expression identified in this analysis have been validated using quantitative RT-PCR, measurement of protein expression and relevant functional assays.

Results: With respect to non-transgenic cells, the transgenic microglia showed 61 differentially expressed genes, most of which were down-regulated. The key pathways affected in the transgenic cells appeared to be those related to the actin cytoskeleton, cellular adhesion, phagocytosis and motility and genes involved in the calcium signalling system. Preliminary validation assays confirm the microarray results at both the mRNA and protein level.

Discussion and conclusion: In keeping with other gene expression profiling studies investigating the cellular pathways affected by the presence of mutant SOD1, our data show a predominant down-regulation of differentially expressed genes. The effects of human SOD1 G93A on the microglial transcriptome provide novel and interesting insights into which cellular properties might be altered in

transgenic cells to hinder their neuroprotective function. The inability of microglial cells to recognize and support damaged motor neurons may prove essential for ALS pathology.

C83 THE 'DOUBLE-EDGED SWORD' EFFECT OF MICROGLIAL ACTIVATION IN SOD1^{G93A} ALS MICE NULL FOR CX3CR1: SPINAL CORD AND CORTICAL PATHOLOGY

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Background: Motor neuron (MN) loss in ALS is believed to be influenced by microglial mediated inflammation. Activation of microglia is normally suppressed by neuronally produced fractalkine (CX3CL1) acting via its receptor (CX3CR1) found exclusively on microglia. Our preliminary findings indicate that knocking out CX3CR1 in transgenic (Tg) mice expressing the mutated human SOD1^{G93A} gene may initially improve MN function but ultimately worsens MN loss in the lumbar spinal cord, resulting in more rapid limb weakness, and early death.

Objectives: 1) To confirm our preliminary findings that SOD1^{G93A} mice without fractalkine receptor (CX3CR1$^{-/-}$) experience more microglial activation, greater MN loss, and more rapid disease progression; 2) To examine whether microglia can be neuroprotective in early disease; 3) To determine if onset of disease is earlier in SOD1^{G93A}-CX3CR1$^{-/-}$ mice; 4) To determine if microglial neurotoxicity is enhanced in cortex – all relative to SOD1^{G93A} mice with one or two copies of CX3CR1 (CX3CR1$^{+/-}$, CX3CR1$^{+/+}$).

Methods: We studied a colony of mice that were SOD1^{G93A}-CX3CR1$^{-/-}$, SOD1^{G93A}-CX3CR1$^{+/-}$, SOD1^{G93A}-CX3CR1$^{+/+}$, and non-Tg SOD1^{G93A}-CX3CR1$^{+/-}$, -CX3CR1$^{-/-}$, with serial behavioral and survival assessments, and histological analysis of spinal cord and cortex in terminal mice at 18–20 weeks.

Results: After 7–9 weeks, limb strength was lower in SOD1^{G93A} mice compared to non-Tg SOD1^{G93A}-CX3CR1$^{-/-}$ littermate controls, and from 15 to 20 wks the decline was more rapid in SOD1^{G93A}-CX3CR1$^{-/-}$ mice for hindlimb ($p<0.01$) and forelimb ($p=0.02$) grip strength compared to SOD1^{G93A}-CX3CR1$^{+/-}$ mice. The male CX3CR1$^{-/-}$ group had the largest decline and accounted for the group differences for hindlimb (-19.9 ± 2.1, estimated slope $\pm$ SE) and forelimb strength (-23.6 ± 3.8) compared to all other groups. However, between 14 and 15 weeks, SOD1^{G93A}-CX3CR1$^{-/-}$ mice were significantly stronger. Disease onset was slightly sooner and survival was shorter in SOD1^{G93A}-CX3CR1$^{-/-}$ mice (18.4 weeks) compared to SOD1^{G93A}-CX3CR1$^{+/-}$ mice (20.1 weeks, $p=0.01$). Microglial reaction was increased, Nissl-stained lumbar cord MNs were reduced, and neuronal injury was greater in the cortex of SOD1^{G93A}-CX3CR1$^{-/-}$ mice compared with SOD1^{G93A}-CX3CR1$^{+/-}$ mice.

Discussion and conclusions: Absence of the CX3CR1 in SOD1^{G93A} mice results in microglial activation which initially enhances MN function. This neuroprotective effect is transient and eventually gives way to worsened neurobehavioral outcomes, greater MN loss, and shorter survival, especially in males. Onset of disease also appears slightly earlier in CX3CR1 null mice. Exaggeration of cortical pathology with degenerating motor projection neurons indicates an extra-spinal effect of microglial neurotoxicity. Ongoing studies at pre-terminal and terminal ages are exploring downstream mediators of microglial neuroprotection and neurotoxicity in an effort to develop pharmacotherapies for ALS that would enhance the former and suppress the latter for improved MN survival and function.

Acknowledgement: Funding was provided by the Robert Packard Center for ALS Research at Johns Hopkins University.

C84 PROTECTION BY WILD-TYPE IMMUNE CELLS: A CENTRAL AND PERIPHERAL EVENT AND THEIR THERAPEUTIC POTENTIAL

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Background: Dominant mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis, a progressive, paralytic disease characterized by loss of motor neurons. To address the question of whether central nervous system microglia and the peripheral immune system are involved in this disease, we utilized PU.1$^{-/-}$ mice, which are unable to develop myeloid and lymphoid cells. Wild-type bone marrow transplants (BMT) into mSOD1/PU.1$^{-/-}$ mice repopulated the central and peripheral immune systems, slowed the loss of motor neurons, prolonged survival, and increased disease duration by 40%. These data demonstrate the importance of the immune system in mediating motor neuron protection.

Objectives: As the results with mSOD1/PU.1$^{-/-}$ mice may have been due to the central and/or peripheral immune systems, we determined whether wild-type microglia, peripheral immune cells, or both, were responsible for the observed neuroprotection.

Methods and results: Using primary motor neuron/microglia co-cultures, mSOD1 microglia were found to be more neurotoxic than wild-type microglia. When mSOD1 mice were irradiated followed by BMT from wild-type or green fluorescent protein donor animals (which replaces the peripheral immune system, but only a few parenchymal immune cells) there was no protection. In contrast

with our earlier study using mSOD1/PU.1$^{-/-}$ mice, when mSOD1/PU.1$^{-/-}$ mice were transplanted with CCR2$^{-/-}$-derived bone marrow, the receptor for MCP-1 involved in immune cell recruitment, the protective effects of wild-type microglia and immune cells were again lost. To determine the contribution of the peripheral immune system to disease, mSOD1 was expressed in the RAG2$^{-/-}$ mice, which lack functional T- and B-cells. Compared with mSOD1/RAG2$^{+/-}$ mice, disease progression was significantly accelerated in mSOD1/RAG2$^{-/-}$ mice.

Discussion and conclusions: These data emphasize the importance of the neuroprotection provided by wild-type BMT in mSOD1/PU.1$^{-/-}$ mice, which involves both the central and peripheral immune systems. The toxic effect of mSOD1 microglia in mice corresponds to toxic effects observed in culture. Furthermore, in the absence of functional T- and/or B-cells, there is an enhanced toxicity, suggesting that T- and/or B-cells may alter the state of microglial activation. The data using CCR2$^{-/-}$ bone marrow suggest that the lack of active recruitment of neuroprotective cells hastens motor neuron injury. These studies are in agreement with the optic (1) or facial nerve (2) injury models suggesting that neuroprotection involves the communication between central microglia and peripheral immune cells. Additionally, our data suggest that following irradiation, wild-type or GFP BMT does not alter disease onset, survival, or duration in mSOD1 mice. Because wild-type peripheral immune cells enter sites of CNS injury, these cells may be manipulated to enhance their neuroprotective attributes and possibly to be used therapeutically.

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C85 OBSERVED ELEVATED CYTOKINES AND MYELOID / INTERFERON-INDUCED (MIFN) SIGNATURE PROTEIN LEVELS IN ALS: A POSSIBLE ALS EARLY DETECTION METHOD

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Background: We have observed that ALS patients have elevated levels of activated macrophages in the peripheral blood and proceeded to investigate the gene expression pattern using microarrays and quantitative RT-PCR. Peripheral blood mononuclear cells from ALS and healthy individuals were found to express a distinct myeloid/interferon-induced (MIFN) transcriptional signature. The MIFN Signature is a characteristic transcriptional response of myeloid cells occurring immediately after cell isolation from the bloodstream. To further evaluate the MIFN Signature, a number of cytokines and genes that were highly up-regulated in ALS patients were selected for analysis of their protein expression.

Objective: To investigate the expression of MIFN Signature proteins and pro-inflammatory cytokines by peripheral blood myeloid cells from individuals with ALS.

Methods: Plasma and whole blood cells were obtained from ALS patients and healthy controls. Mononuclear cells were purified from blood and incubated in cell culture medium for 24 h at 37°C. Tissue culture supernatants (TCS) from cultured mononuclear cells were tested for secreted cytokine and MIFN Signature proteins by ELISA, as was plasma from ALS patients and controls. Cell surface and intracellular expression of MIFN Signature proteins were analysed by flow cytometry.

Results: In TCS from ALS patient mononuclear cells, TNF-alpha levels increased from 97 pg/ml to 159 pg/ml within 3 h before decreasing to 40 pg/ml after 24 h; TNF-alpha levels from controls remained at 2 pg/ml through all time points. IL-6 protein levels in ALS TCS increased 7.8-fold in 24 h, while the increase in IL-6 levels from healthy individuals were negligible. The MIFN signature proteins, PI3 (Elafin) and IL-1 receptor antagonist (IL-1RN), increased 53- and 67-fold, respectively, after 24 h in TCS from ALS patients but not controls. Mean plasma protein levels of PI3 were approximately 2-fold higher in ALS patients than in healthy individuals ($p < 0.05$). Mean IL-1RN levels were reduced in ALS patients relative to controls. TRAIL and IL-6 plasma levels were similar in both groups. Expression of 10 MIFN Signature proteins was evaluated by flow cytometry and all 10 proteins were expressed primarily in polymorphonuclear cells and monocytes. Of the MIFN Signature proteins evaluated, IL-1RN, FPRL1, and Chitinase 3-like 1 (CHI3L1), were found to have a higher mean fluorescent staining at both 0 and 24 h among ALS patients relative to controls.

Conclusions: Peripheral blood myeloid cells from patients with ALS are committed to a pro-inflammatory program dominated by secretion of TNF-alpha and IL-6 prior to leaving the bloodstream. Additionally, peripheral blood myeloid cells from ALS patients express a distinct protein profile that can be employed to identify individuals with ALS. This can benefit both the patient and the medical community by allowing for earlier treatment of this neurodegenerative disorder.

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SESSION 10B RESPIRATORY MANAGEMENT

C86 RESPIRATORY PHYSIOLOGY AND MANAGEMENT STRATEGIES

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ALS/MND is a progressive disease for which no satisfactory treatment presently exists; muscles supplied by the degenerating nervous system develop weakness and this process affects the respiratory muscles in the same way as limb muscles. Thus respiratory muscle weakness is a common feature in ALS/MND although it is the presenting feature in just 3–5% of cases. In the respiratory management of ALS/MND the following issues require consideration:

1. *Identification of respiratory muscle involvement*. Respiratory muscle weakness is suggested by breathlessness particularly if accentuated by lying flat. Nocturnal hypoventilation (and CO_2 retention) is suggested by morning headache and daytime sleepiness. The simplest investigation to confirm or exclude respiratory muscle weakness is the vital capacity but this test is unreliable in advanced disease and recent data suggest that the maximal sniff nasal inspiratory pressure is more sensitive and specific for the identification of hypercapnia (1).
2. *Screening for respiratory failure*. Frank respiratory failure is usually preceded by hypoventilation during sleep. This can be evaluated by polysomnography but transcutaneous O_2/CO_2 monitoring may also be used to identify incipient respiratory failure. Alternative measures include arterial bicarbonate or serum chloride (2).
3. *Treatment*. Following earlier cohort studies it has become established, using a randomized controlled trial, that non-invasive ventilation extends life and improves quality of life in patients with non-bulbar ALS/MND (3), although the advantages are less clear-cut for patients with bulbar disease who tolerate NIV less well. Adjunctive strategies including physiotherapy and mechanical insufflation-exsufflation (4) may also be useful in patients who experience problems with sputum clearance. Although the burden of care experienced by caregivers increases as MND progresses, NIV does not significantly increase most aspects of caregiver burden (5).

Some issues are unresolved and may vary between healthcare systems. These are if and when to use tracheotomy ventilation and management of end of life care.

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C87 CLINICAL CHARACTERISTICS OF RESPIRATORY ONSET ALS AND RESPIRATORY SYMPTOMS AT DIAGNOSIS IN PATIENTS WITH ALS

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Background: ALS can have respiratory muscle weakness as the initial symptom. When the disease begins with respiratory failure, the diagnosis can be difficult to make, and survival may be shorter.

Objective: To describe the frequency, demographics, clinical features and survival of patients with respiratory onset ALS and respiratory symptoms at diagnosis.

Methods: We retrospectively reviewed 502 consecutive charts of patients evaluated at our outpatient ALS Center with IRB approval. All met El Escorial criteria for the diagnosis of ALS. Patients were segregated into respiratory onset (RO) and non-respiratory onset groups. The non-respiratory onset group was further subdivided by the presence (RS) or absence (NR) of respiratory symptoms at the time of diagnosis. Information regarding demographics, site of onset, symptoms at diagnosis, age, gender, EMG findings, ventilation, and death were obtained. Information was obtained by phone for those patients not evaluated during the past year. Data were analysed using unpaired *t*-tests.

Results: Two per cent (11/502) of patients were in the RO group, 8% (42/502) in the RS group, and 90% (449/502) in the NR group. The RO group consisted of 81% (9/11) males compared to 52% (22/42) in the RS group and 56% (249/449) in the NR group. Mean age of onset in the RO (65 ± 6 years) and RS groups (65 ± 9 years) were significantly higher than the non-respiratory onset group (60 ± 13 years, $p=0.024$, $p=0.021$). The most frequent symptom was dyspnea on exertion in the RO (7/11, 64%)

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and RS (19/42, 45%) groups. Only one patient had respiratory failure requiring invasive ventilation as the first sign. Mean time from onset to diagnosis in the RO group was 14.4 ± 9 months and was not statistically different from the NR group (15.7 ± 15 months, $p=0.76$). At the time of diagnosis, most patients with respiratory onset had arm (5/11) or leg (4/11) weakness. Only two of 11patients had bulbar weakness. EMGs of the parapsinal muscles were available in 65% of patients but there was no difference in involvement of the thoracic paraspinal muscles in the RO group (60%) compared to the NR group (68%). In the RO group, 73% used NIPPV (mean time from onset to NIPPV=8.7 ± 5 months) and 63% used NIPPV (mean time from onset to NIPPV=21.1 ± 18 months) in the RS group. Five of 11 patients in the RO group died, two were maintained on mechanical ventilation and one was lost to follow-up. Mean survival from symptom onset to death in the RO (30.4 ± 6 months) and RS (27.8 ± 19 months) groups was not statistically different from the NR group (30.6 ± 19 months, $p=0.98$, $p=0.48$).

Conclusion: These data suggest that respiratory onset ALS is rare and occurs more commonly in males. Respiratory onset and respiratory symptoms at diagnosis occurred in older individuals and dyspnea on exertion was the most frequent initial symptom. Onset to diagnosis and survival was not different from non-respiratory onset ALS.

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C88 THE USE OF MAXIMAL INSPIRATORY PRESSURE (MIP) AS AN INDICATOR FOR EARLY INITIATION OF NIPPV IN ALS PATIENTS

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Background: The early use of non-invasive positive pressure ventilation (NIPPV) in ALS patients is associated with improved quality of life and survival (1,2). Current AAN and American College of Chest Physicians guidelines recommend initiation of NIPPV when the forced vital capacity (FVC) decreases below 50% of predicted (3,4). However, supine FVC and maximal inspiratory pressures (MIP) are considered more sensitive indicators of diaphragmatic muscle weakness and better predictors of survival than upright FVC (5–7). In those patients unable to comply with a supine FVC measurement due to orthopnea, MIP is a useful and sensitive indicator of respiratory dysfunction and for early initiation of NIPPV, even in those patients with FVCs above 50%.

Objectives: To determine the prevalence of diaphragmatic dysfunction as measured by upright MIP in ALS patients with FVCs above 50% and 70%. To determine the relationship of MIP and FVC and the association with other measures of respiratory dysfunction, disease disability and progression.

Methods: One hundred and fifty-two patients with a diagnosis of definite or probable ALS were studied at the Methodist Neurological Institute. Forced vital capacity and maximal inspiratory pressures were measured and studies were interpreted by the clinic pulmonologist for all patients. Disease disability was assessed with the Appel ALS rating scale at time of testing. EMG studies of the diaphragm were performed for a sub-set of patients. Correlation analyses of study variables were performed with linear regression and χ^2 testing.

Results: One hundred and twenty-six ALS patients had both FVC and MIP measured during the study period (39% females, 31% bulbar). The average AALS score was 81 ± 28 at time of study and disease duration was 35 ± 36 months. FVC and MIP were 72 ± 26 and 46 ± 26, respectively. FVC and MIP were significantly correlated ($p<0.0001$; $r=0.63$), yet 61% with FVCs>70 had MIPs<60. For patients with FVC$\leqslant 70$, 98% had MIPs<60. Of patients with FVC$\geqslant 50$%, 26% of patients had MIPs<60, whereas of those with FVC$\leqslant 50$, 93% had MIPs<60. Both FVC and MIP correlated with AALS score at time of measurement, but only the FVC was significantly associated with bulbar onset ALS ($p=0.025$). Diaphragmatic denervation was present in 7% of patients. Twenty-five per cent of these had MIPs<60 and FVCs>70.

Conclusions: Respiratory dysfunction as measured by MIP is present in one-quarter of ALS patients who would not otherwise meet current guidelines for NIPPV initiation (FVCs>50). Furthermore, more than 50% of patients with FVC>70 had evidence of diaphragmatic weakness as measured by a MIP<60. Thus, MIP is a more sensitive indicator of respiratory dysfunction in ALS and provides the basis for earlier initiation of NIPPV.

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C89 USE OF NIPPV COMPLIANCE AND EFFICACY DATA

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Background: The use of non-invasive positive air pressure ventilation (NIPPV) has benefits for people with ALS (1). Both quality, as well as quantity of life can be improved. However, there is not an ALS standard of care for management of NIPPV therapy. It has been reported that the use of frequent monitoring of spirometry is paramount in the timeliness of initiation of NIPPV (1). NIPPV devices contain internal pressure monitors (pneumotachographs) that measure parameters of both compliance (numbers of hours used) and efficacy (minute ventilation, leak, respiratory rate, tidal volume, and apnea/hypopnea index). These data are available on current commercial home NIPPV devices. The interpretation of these data and its relevance to clinical management of ALS patients has not been described. Frequent home monitoring of NIPPV devices may help standardize NIPPV therapy for people with ALS and improve respiratory function.

Objectives: To review data collected from home NIPPV devices; to identify how often the data obtained prompted a change in the therapy and to describe the types of changes made.

Methods: Compliance and efficacy data were obtained from a retrospective review of nine people with ALS using NIPPV. The routine for collecting the data is a minimum of three months. Changes are made more frequently with reported symptoms. This information included how often data were obtained and how frequently those data influenced changes in therapy. Therapy was managed by the pulmonologist in the ALS Center. An effective breathing pattern was determined by calculating the shallow breathing index (respiratory rate/tidal volume) which has been shown to predict successful liberation from mechanical ventilation (2). The ALSFRS-R score, FVC, tidal volume, compliance data, and patient's disease status were all collected to describe patient sample characteristics.

Results: NIPPV was initiated when FVC reached 50% (mean=19 months). Compliance and efficacy data were collected an average of every 2.5 months. Four changes in therapy were prescribed after data review. Oxygen was added due to low saturation. Conversion to a new device occurred because of a high apnea/hypopnea index, high respiratory rate or high total usage hours. Changes in pressures or inspiratory time minimum or maximum settings were made due to low tidal volumes or high shallow breathing index results. Increases were made to back up rates because of high apnea/hypopnea index results. A change in therapy occurred 42% of the time when data were collected.

Discussions and conclusions: Compliance and efficacy data interpretation can assist healthcare professionals in maximizing NIPPV therapy for people with ALS. Managing the shallow breathing index has proved helpful. Further studies are needed to confirm the reliability of this index to improve respiratory function and reduce the work of breathing. Patients may benefit from monitoring data at a minimum of every three months, or as frequently as symptoms are reported. Further studies may indicate more frequent monitoring is needed.

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C90 RESULTS OF DIAPHRAGM PACING IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): DECREASING THE DECLINE IN RESPIRATORY FAILURE AND INCREASING DIAPHRAGM MOVEMENT

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Background: ALS patients develop respiratory insufficiency which is ultimately responsible for the majority of deaths. Therapeutic electrical stimulation has been shown to maintain the strength of other peripheral muscles in ALS by maintaining physiological activity, contractile properties and calcium levels. Motor units can be compensated for by collateral axon sprouting and the rate of sprouting increases with electrical stimulation. The laparoscopic diaphragm pacing system (DPS) is a low-risk outpatient system to stimulate and condition the diaphragm.

Objective: To evaluate in a phase I trial that the DPS system can be safely implanted and used for conditioning the diaphragm in ALS patients. Secondary objectives are to show that life threatening respiratory muscle dysfunction may be delayed with diaphragm pacing.

Methods: Patients diagnosed with ALS and with a forced vital capacity (FVC) above 50% predicted were eligible. Each patient had three lead-in assessments, at four-week intervals prior to implantation, with pulmonary function tests, fluoroscopic evaluation of diaphragm movement, speech phonation times, ultrasound analysis of diaphragm thickness, phrenic nerve conduction tests and quality of life tests. Patients underwent laparoscopic mapping of their diaphragm to locate the phrenic nerve

motor points and two electrodes were implanted in each hemidiaphragm. Two weeks after surgery, stimulus/output characteristics of each electrode were determined. The patients then conditioned the diaphragm with five 30-min sessions of therapeutic electrical stimulation per day. Patients were similarly assessed post-operatively.

Results: Eight patients have been safely implanted and have started on diaphragm conditioning with the DPS system with no adverse events. There have been no deaths or need for tracheostomy. In all patients, more fluoroscopically observed diaphragm excursion occurs with diaphragm stimulation than under maximal patient control. The first four patients (average FVC at implantation was 49%) have an average 12-month follow-up. Their monthly decline in FVC went from 4.1% pre-implant to 1.4% post-implant. Patient 3 utilizes DPS continuously to augment respiration and has no diaphragm movement without DPS. Phonation time has improved and muscle thickness (mass) has increased with DPS. With the exception of patient 3, there has been no change in the respiratory subscore of the ALSFRS-R, although overall score has declined. There has been no decline in the SF-36 emotional domain quality of life scores although the SF - 36 physical function has declined.

Conclusion: The diaphragm pacing system can be safely implanted and utilized in patients with ALS. There has been a documented decrease in the decline of respiratory failure which leads to an increased survival. The ability to cause more diaphragm movement with DPS is a surprising and beneficial finding. This may be best explained by intact phrenic nerve motor neurons that are no longer controlled by the medullary respiratory center, cerebral cortex, or central or peripheral chemoreceptors, but can be stimulated with DPS. DPS may also have a trophic effect on increasing the survival of these motor neurons. DPS also converts the remaining motor units to usable slow twitch oxidative units. These findings of DPS may lead to improved night time ventilation, decreased posterior lung lobe atelectasis and subsequent pneumonia.

C91 PHRENIC NERVE PACING TO IMPROVE RESPIRATORY FUNCTION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Surgical implantation of electrodes for direct stimulation of the phrenic nerve has been used as an alternative to invasive ventilation in patients with spinal cord and nerve injury. Phrenic nerve function has been enhanced and nerve viability preserved via programmed electrical pulses delivered to the nerve and then transmitted to the diaphragm muscles. To date, use of this procedure has not been reported in patients with motor neuron disease due to the progressive degenerative nature of the disease.

Objective: To describe our experience with surgical implantation of a phrenic nerve pacemaker in three patients with advanced ALS and significant respiratory compromise.

Methods: The number of hours per day of non-invasive positive pressure ventilation was measured prior to and after implantation of a bilateral phrenic nerve pacemaker. A receiver was implanted in the upper chest and connected to electrodes placed around the phrenic nerve in the chest. Forced vital capacity, Bipap dependence and the patient's subjective assessment of their respiratory function were recorded. A pre-implantation phrenic nerve conduction study was performed to assist in our selection of appropriate patients.

Results: Two of our three patients noted significant benefit within 48 h from onset of pacing. Tolerance for increasing time on the pacemaker was favourable and in both patients who benefited, significant improvement in their quality of life and respiratory function resulted. Forced vital capacity had dropped aggressively in the ten months preceding implantation in all patients. After use of the pacemaker for at least one month, the rate of FVC decline was abated and respiratory function stabilized.

Conclusion: Despite the logical contraindication to aggressive surgical intervention with a phrenic nerve pacemaker, the procedure was beneficial in two patients we studied with respiratory impairment due to ALS. Decline in forced vital capacity was stabilized, use of BiPAP decreased and subjective assessment of functional ability greatly increased. In appropriately selected patients with some preserved phrenic nerve conduction and precipitous respiratory decline, surgical implantation of a phrenic neve pacemaker may be an appropriate alternative therapy. Implanted patients were able to increase time off Bipap and prolong latency to initiation of invasive ventilation.

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SESSION 11 JOINT CLOSING SESSION

C92 LOU GEHRIG, *RAWHIDE* AND 1938

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Background: Lou Gehrig was one of the most talented baseball players of all time; yet he is also remembered for the disease, amyotrophic lateral sclerosis (ALS), that took his life and still bears his name as its eponym. There is considerable speculation about when his symptoms began; some authorities believe that Gehrig had signs of ALS when he starred in the film, Rawhide, in January 1938, which would mean that he played the entire 1938 season with symptomatic ALS. Uniform logos have been used to date the year of photographs taken of Gehrig while he was playing baseball. We re-examined the film and photographs, to determine when he first displayed evidence of ALS.

Objective: To determine whether Gehrig had signs of ALS in Rawhide, to assess his physical function in the film, and to describe photographs taken of Gehrig from 1937 to 1939.

Methods: Specific scenes from Rawhide were chosen to grade Gehrig's hand, arm, leg and bulbar function using the ALS Functional Rating Scale (ALSFRS). Separately, we checked with the Baseball Hall of Fame to ascertain the date photographs were taken, and scrutinized them for evidence of hand atrophy or leg weakness.

Results: Gehrig did not display visible signs of weakness in Rawhide. Bulbar function, handwriting, handling utensils, walking, climbing stairs, and breathing were normal. His ALSFRS score, modified to account for limitations of on-screen activities, was normal (score=32/32). There was no visible hand atrophy or limb weakness. He lifted an adult man overhead (time=22:00), threw billiards balls with force and precision (22:00), stood from a seated position while clapping his hands (41:50), and leapt over a chair (57:56). Close-up shots of his hands showed normal muscle mass (12:10, 24:40). Photographs from the spring of 1938 show normal hand bulk and leg function. A photograph of Gehrig mid-swing taken in September 1938 implies good leg strength. The New York Yankees wore logos in 1938 that were labelled with the year 1939 to advertise the 1939 World Fair, while the uniforms displayed a centennial patch during the 1939 season.

Conclusions: Examination of *Rawhide* provides evidence that Gehrig functioned normally in January 1938. Several scenes illustrate exceptional strength and coordination and no hand atrophy or leg weakness. Photographs taken during the years 1937–1939 imply that physical signs first appeared after May 1938. Physical abnormalities of his hands prior to mid-1938 were more likely due to bone injuries. Uniform patches, previously misidentified in documenting dates, can be used to discriminate between photographs taken of Gehrig in 1938 and 1939. We conclude that there was no definite evidence of ALS in photographs taken of Gehrig prior to mid- season 1938. The presentation will include video footage to document function and photographs to construct a timeline for hand atrophy.

C93 ANTIOXIDANTS AS THERAPIES FOR NEURODEGENERATIVE DISEASE

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The major neurodegenerative diseases affect different parts of the brain and have different pathologies and prognoses. However, they have several common features, including increased oxidative damage, mitochondrial dysfunction, defects in the clearance of unwanted proteins, iron accumulation and some involvement of inflammation and of excitotoxicity. The link between these events will be discussed, and a unifying hypothesis of the origins of neurodegeneration will be presented. The prospects for prevention and treatment of neurodegenerative diseases by the use of antioxidants will be assessed.

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THEME 1 RESEARCH TO IMPROVE STANDARDS OF CARE

P1 THE EFFECT OF MUSIC THERAPY FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: The definitive treatment for most intractable neurological diseases has not been established. The patients have to struggle with the disease for a long time. Since the psychological conditions of amyotrophic lateral sclerosis (ALS) are extremely distressing and stressful for patients, palliative care such as music therapy could be supportive. The aim of this study was to investigate the effects of music therapy for ALS patients who have low willingness and mental depression.

Method: Thirteen patients with ALS in a long-term care ward were enrolled in this study. They were divided into two groups: the therapy group (11 cases, 63.91 ± 10.9 years of age) and the non-therapy group (8 cases, 66.0 ± 11.2 years of age). Passive music therapy, appreciation of the patient's requested music, was provided for 30 to 45 minutes twice a week. We had eight sessions for each patient in the therapy group. We evaluated the Japan Stroke Scale-Emotional Disturbance Scale- (JSS-D, JSS-E), and Vitality Index (VI). The serum level of an activity of NK cells was also measured before and after the music therapy.

Results: In the therapy group, the score of JSS-D, JSS-E was significantly decreased, and the score of VI increased significantly after the music therapy. The activity of NK cells also increased after the sessions. The changes of these scores and NK cell activity were not observed in the non-therapy group.

Conclusion: Since the music therapy improved psychological conditions as well as immunocompetence, it may have beneficial effects as palliative care for individuals with ALS.

P2 COPING STRATEGIES IN LIFE WITH ALS/MND

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Background: It is now possible to live with ALS for some years after respiratory failure has occurred by use of mechanical ventilation. To decide to live with permanent mechanical ventilation means that the patient and his or her family might have to live with the ongoing development of the disease and increasing disabilities for several years.

Knowledge about coping strategies for the ALS patient and the family to live with the ongoing losses of body functions and new problems from the disease, might be a resource for the professionals to give the right treatment and support at the right time.

Objectives: To acquire more knowledge about:

- Which personal factors are important to ALS patients and their spouses to cope with the physical, psychological, economic and practical problems that are a consequence of having ALS
- Which coping strategies can help people to have a good life despite ALS

Methods: Interviews were performed in ALS patients' homes from two counties in Denmark based on the following themes: 1) The impact of the disease on daily life; 2) Information obtained by the patient and the spouse about the disease and its consequences; 3) The former life of the patient and family; 4) Practical and personal help; 5) Expectations for the future, including choice of life-prolonging treatment. At every interview the patient completed a Sense of Coherence (SOC) questionnaire, and at the first interview the patient and the spouse individually completed SF36. Analysis of the interviews was based on theoretical aspects of coping strategies.

Results: Twenty-three patients (16 males and 7 females) were included from January 2004 to November 2005. Only one female lived alone. All participants were interviewed once and 17 were interviewed a second time. Five widows were interviewed within six months after their husbands had died. Ten participants have died and two live with permanent mechanical ventilation.

Conclusion: Analysis of the interviews is not finished but preliminary conclusions are:

- Coping strategies among patients with ALS are similar to the way they coped with problems before the disease.

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Knowledge of the previous life of the patient is therefore a good resource for professionals supporting ALS patients.

- Finding life meaningful and manageable for ALS patients seems to depend on what the individual patient considers to be important to preserve quality of life. Information from the professionals, degree of disability and dependence on physical and practical help are important, but not crucial for coping with the disease.

P3 PATIENTS' EXPERIENCES WHEN DIAGNOSED WITH ALS

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Background: Disclosing the truth to patients with ALS is a daunting task for physicians. Today, many agree that the diagnosis should be announced directly to patients, preferably in the presence of patients' family member(s). Now the issue is when and how to tell the truth and there is no consensus of opinion. Probably there will be no single answer. In order to perform this difficult task in better way, we must learn what they feel and experience when they are confronted with the bad news.

Objectives: To understand the psychological state of patients when they are diagnosed with ALS. A better understanding of their emotional experiences will help us in providing holistic care and will enhance the QOL of patients.

Methods: Semi-structured interviews were conducted with 18 patients (including seven ventilated). They were asked to report what they felt when diagnosed with ALS and any other comments on their experiences. Those who were not told the diagnosis reported their feelings when they knew the truth by other means. The purpose of the study was explained and they agreed to cooperate. Male: female ratio was 2:1 and mean age was 53 years (range 67–34 years).

Results: Fourteen patients were told the diagnosis and four were not. Various feelings were reported such as shock, disbelief, surprise, anger and anxiety. Five accepted the fact unperturbedly. Seven reported that they ''felt somewhat relieved that the disease was identified''. Patients often expressed that ''unidentified suspended state is harder than having an intractable disease''. Those who were indirectly told that they had ALS felt inadequate. Satisfied patients reported that their doctor was a good listener, sincere, and not too pessimistic but hope-giving. Many emphasized the necessity of psychological care for themselves as well as for their family.

Discussion and conclusions: As all the patients could recall their emotional experiences clearly, it was an unforgettable life event for them. Identity issue has the greatest meaning. To live with ALS, they need truth, hope, psychological care and opportunity to express their feelings.

P4 PEOPLE WITH ALS/MND AS AUTHORS: WHO IS WRITING ABOUT LIFE WITH ALS/MND?

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Background: Health professionals are increasingly recognizing the value of illness narratives to inform them about the personal experiences of living with illness. Illness narratives are obtained directly from patients during interviews, or from their writing published in print medium or posted electronically on the internet. To date no published work has sought to investigate the use of illness narratives by people with ALS/MND.

Objectives: To identify published and unpublished illness narratives written by people with ALS/MND and compare the genres to establish the characteristics of the authors.

Method: A comprehensive search strategy was adopted to identify and locate electronic and print medium narratives written by people with ALS/MND. Following this scoping exercise, identified literature was assessed according to inclusion/exclusion criteria to obtain a comprehensive sample of 152 narratives (88 print narratives, 64 electronic narratives) to subject to analysis. Demographic details including sex, geographical location of author, occupation, age at diagnosis, decade of diagnosis and publication were recorded where available and comparisons made across the genres.

Results: Men wrote more than 60% of all narratives and within each genre produced more illness narratives than women. Although people from a number of countries worldwide have written about their illness experiences, over 60% of all narratives emanate from the USA. People from a wide variety of occupational backgrounds documented their illness experiences; 62% of authors specified their occupation. Twenty-five per cent of print medium accounts were written by professional writers (academics/journalists); people in office-based occupations (supervisor/clerical) wrote 21% and management/business people wrote 16% of printed narratives. Electronic narratives, in contrast, were dominated by writings from unskilled/manual workers and members of the emergency services/armed forces, who each produced 16% of the internet material.

The author's age at diagnosis was included in 55% of all narratives (range 20–76 years); those aged under 40 years of age at the time of their diagnosis produced more electronic (47%) than print medium (31%) narratives; while those aged over 41 years at diagnosis produced more print narratives (68%) than internet narratives (50%). The experiences reported within both genres spanned the decades from the 1960s; however, 75% of authors, regardless of the genre they adopted, received their diagnosis during the 1990s.

Discussion and conclusions: This study demonstrates the extent and availability of written narratives about the personal experience of living with ALS/MND. A substantial quantity of both published and unpublished personal illness narratives from the 1960s onwards has been identified. A broad cross-section of people with ALS/MND, including those not normally associated with publication of personal stories, have documented their illness experiences within print and electronic mediums. The study has identified an unparalleled collection of personal illness narratives about life with ALS/MND that will help to illuminate what it means to be diagnosed with the illness.

P5 DISASTER PREPAREDNESS FOR THE FAMILIES OF PEOPLE WITH ALS

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Background and objectives: The purpose of this study was to learn about current evacuation plans for ALS patients with invasive ventilators at home in case of natural disasters. At least 30% of ALS patients wear invasive ventilators and 30% of them stay at home in Japan, where typhoons kill about 200 people a year and a large earthquake occurs every ten years. The Japanese government has been considering its role and encouraging local government to prepare shelters and plan for people with special health needs in case of natural disasters. However, the plans are currently mainly focused for hospital settings and shelters, not for ALS patients at home.

Methods: Semi-structured interviews were conducted with five ALS patients with ventilators at home and seven family members. The conversations with the patients were helped by family members using transparent boards. The family members included two wives, one elder sister, one nephew and two daughters. The interviews were fully recorded with an IC recorder and transcribed to analyse the contents.

Results: 1) Every patients had at least one emergency bag for power cuts, and had the TV sets at lower places to avoid hitting the patients during earthquakes; 2) Every patient knew the place of evacuation that the local government had prepared for the residents, but none of them had ever tried to reach the place; 3) Everybody said that they would not move from the house until a rescuer came to help; this is because usually one family member, sometimes a young child, stays with the patient and there would be nobody to go and call for help. It also takes about two or three hours to prepare to go out; 4) Two patients had experienced no offer of any help by local government in the case of a minor natural disaster.

Discussion: The following preparations are suggested to be required for ALS patients with ventilators at home, in case of natural disasters; 1) emergency drills and a hotline to the emergency office to ask to move the patients to a safe place; 2) nursing assistants if the regular assistants are unable to come in case of a major disaster. 3) care for young children of patients because they are possibly forced to work as the carer in an emergency.

P6 ALS PATIENTS AND THE ROLE OF THE COORDINATOR FOR PATIENTS WITH INTRACTABLE NEUROLOGICAL DISEASES

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Background: More than a half of the 47 prefectures of Japan have posted one or more coordinators for patients with intractable neurological diseases. Their activities have so influenced health care professionals, patients and family that they have been convinced that coordinators can help patients' domiciliary care. However, there are many difficulties in finding suitable long-term care facilities or hospitals to take responsibility for respite care. In particular it is very difficult for patients with amyotrophic lateral sclerosis (ALS) who have received tracheostomies and are on mechanical ventilation to find such a place. In our prefecture, a coordinator for patients with intractable neurological diseases has been posted at the university hospital for three years, as an adjunct to the staff at the Department of Health and Welfare of the prefectural government. The coordinator is a registered nurse with more than 15 years' experience and has accepted many consultations and negotiations. However, it is unclear whether or not all cases that need consultation with the coordinator are able to come to her.

Objectives: The aim of the present study is to investigate the ratio of cases which were brought to the coordinator compared to patients with intractable neurological diseases finding a suitable facility from the public health nurse, at all the nine public health institutes in the prefecture in the 2005 fiscal year (from April 2005 to March 2006) and to review the issues from the viewpoint for ALS.

Methods: The study design was cross-sectional and the survey was conducted using a questionnaire that asked the public health nurses at the public health institutes who had responsibility for the care of the patients registered for the specified diseases by the Ministry of Health, Welfare and Labour of Japan. The survey was carried out in April 2006.

Results: The questionnaires were retrieved from all the nine public health institutes. Except for one, all the cases where the public health nurses recognized the necessity of coordination in the 2005 fiscal year were referred to the coordinator. There were 40 case records with the coordinator in this period. The records consisted of 24 ALS and 16 cases of other diseases. The ratio of ALS in the cases was disproportionately and significantly high for the prevalence rate in this period.

Discussion and conclusions: The present study has confirmed that most of ALS cases recognized by public health institutes were referred to the coordinator in the 2005 fiscal year. In addition, these findings suggest a possibility that there may be more ALS cases that were not recognized by the public health nurses or were not registered as an intractable neurological disease for any reason.

P7 THE ACTIVITIES OF MEDICAL HEALTH CARE NETWORKS FOR SERIOUSLY INTRACTABLE NEUROLOGICAL DISEASES IN JAPAN

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Background: Ten years ago there was no public care network for seriously intractable neurological diseases in the Fukuoka region of Japan, making it extremely difficult to find chronic care hospitals for such patients. Thus, the Prefectural Medical Health Care Network for seriously intractable neurological diseases was initiated in 1998 to improve the care for seriously intractable neurological diseases, especially ALS.

Objectives: To clarify the problems and benefits of the Prefectural Medical Health Care Network for seriously intractable neurological diseases in the Fukuoka prefecture in Japan for the past eight years.

Methods: We investigated ALS patient transfers, care conditions, hospital medication, problematic cases, and analysed the system management.

Results: The network now consists of 15 basic cooperating hospitals, and 105 general cooperating hospitals. The total number of patients with seriously intractable neurological diseases who were referred to the network in the last eight years was 277, 163 of whom were ALS patients, and 75 were on mechanical ventilation. Of these, 253 were successfully transferred to chronic care hospitals or referred to regional visiting physicians through the network. Cooperating and non-cooperating hospitals accepted 157 and 58 patients, respectively, while 38 were referred to visiting physicians. Two medical care coordinators managed the transfer of the network's patients based on information on the patient's condition and the capacity of cooperating hospitals, using information about the number of available beds in cooperating hospitals. Each year we investigated the hospitalization and home care environments of these ALS patients. When the network was started, 61% of the transfers were declined by hospitals, which reduced to 38% in 2005 as the network became accepted. The number of medical care consultations has increased year by year, and reached 17,652 in the eighth year. The main issues for these consultations were to make hospital arrangements for a patient. To resolve these issues, care conferences for patients and their family members were held with the attending physician. Additionally, a comprehensive care network for each patient that included hospital management and also home, psychological and urgent care, was constructed. For any emergency problem in the system, home visiting physicians were selected from the enrolled doctors and hospitals. Three times a year we had open meetings with the network's participants for training and information exchange about cases with intractable neurological disease. These workshops and hospital visits considerably encouraged co-medicals and social workers.

Discussion and conclusions: Comprehensive long-term care is necessary for Japanese ALS patients because one-third are on mechanical ventilation. Despite this, it is still difficult to secure available beds for such patients who require long-term care. A more efficient system needs to be constructed and more information provided to surrounding hospitals and home doctors.

P8 THE PRESENT STATUS AND PROBLEMS WITH UPPER EXTREMITY ORTHOSIS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Upper extremity orthosis is able to help patients who have a muscle weakness of proximal upper extremity muscles in the activities of daily living (ADL). There are some studies on the use of orthosis in patients with duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA) and cervical spinal cord injury (SCI), but studies about their use in ALS patients are few. However, upper limb impairment is present in all of these conditions. Thus, it is suggested that orthosis is able to be adapted to ALS as well as DMD, SMA and SCI.

Objective: The purpose of this study was to examine the present status and problems about adaptation of upper extremity orthosis in patients with ALS who have a weakness of proximal muscle of upper limbs.

Methods: Six patients who were diagnosed with ALS were included in this study. Inclusion criteria were: 1) at least one muscle in the shoulder girdle or shoulder or elbow was below a grade of poor or better in manual muscle test (MMT); 2) passive range of motion (ROM) in shoulder flexion and an abduction was 0–90 degrees, internal rotation was 0–90 degrees, and elbow flexion was 0–110 degrees; 3) stability and tolerance for sitting in a wheelchair for more than 30 minutes.

As an outcome measure, the following three items were collected: 1) Acquired ability or changed ability by introduction of upper extremity orthosis; 2) the period over which patients could use the orthosis; 3) reasons that patients were not able to use the orthosis.

Results: 1) Acquired ability or changed ability: four patients could eat, and one patient could input a keyboard independently. One patient could not adapt, because she had dementia. 2) Time period: all patients could use only short term, between one to four months. 3) Reasons for withdrawal: two patients could not use due to decreased muscle strength (progression of disease), and the welfare system in Japan did not permit it in another three patients.

Discussion and conclusions: For patients with ALS, it was suggested that both ADL and QOL were improved by the use of upper extremity orthosis, but the period in which it can be used is limited to the short term due to the rapid progression of the disease. It may be effective for patients with ALS to introduce upper extremity orthosis to improve ADL and QOL, but it is necessary to introduce it as soon as possible. We have to clarify the standard of adaptation of orthosis and also discuss the welfare system for ALS.

P9 ALS PATIENTS AND PRESSURE SORES ON ADMISSION AT A UNIVERSITY HOSPITAL

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Background: Relatively low incidence rates of pressure sores in patients with amyotrophic lateral sclerosis (ALS) have been noticed and some specific skin changes, both morphological and biochemical, were revealed to account for the resistance of ALS patients' skin against pressure sores. However it is still unclear whether or not the incidence rate of pressure sores in patients with ALS is less than in other neurological conditions.

Objectives: To review the incidence rates and outcomes of pressure sores in patients with neurological diseases on admission to our hospital, and compare the rate of ALS with those of other diseases.

Methods: All the 595 patients who had been admitted to the neurology ward of our University Hospital for two years from 1 April 2004 to 31 March 2006 were included in this survey. One of the authors, TH, a wound-ostomy-continence specialist nurse (WOC) has evaluated and treated any skin lesions of all the patients on the neurology ward since 2004. Transverse analysis from the medical and nursing records was performed, particularly focusing on the differences among patients with ALS, Parkinsonism and other neurological disorders. JMP ver. 5.1 (SAS Institute Inc.) was used for statistical analysis.

Results: Seventeen patients (13 males and 4 females, age 33–87 years, average 70.3 ± 15.5 years old) had pressure sores on admission: three of 41 ALS patients (7.3%), five of 126 Parkinsonism patients (4.0%) with Parkinsonism (66 Parkinson's disease, 15 progressive supranuclear palsy, 24 multisystem atrophy, eight dementia with Lewy bodies, five corticobasal degeneration, and eight other Parkinsonism). A proportional analysis by χ^2 test failed to show a significant difference of incidence rates of pressure sores in ALS patients. The patients with ALS who had pressure sores had some complications other than motor symptoms such as cerebrovascular disorder and cognitive impairment together with some problems in home care, similar to the pressure sores in other neurological diseases. None of the patients developed a new pressure sore after admission. Most of 17 patients had light or moderate pressure sores and relatively good improvements during their hospital stay.

Discussion and conclusions: This preliminary survey failed to reveal a large number of cases with pressure sores. One of the reasons for relatively low incidence rates of pressure sores may have been due to earlier cases admitted to a teaching hospital, and good care at home or long-term facilities for patients with neurological diseases prior to admission. However, we would like to stress that pressure sores can occur in ALS patients, particularly in conditions where complications other than motor neuron symptoms occur or the patients are put in poor hygiene circumstances.

P10 FATIGUE IN INDIVIDUALS WITH MND/ LATE EFFECTS OF POLIOMYELITIS: WITH OR WITHOUT POST-POLIOMYELITIS SYNDROME (PPS)

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Introduction: Paralytic poliomyelitis is an infectious viral disease that mainly affects children and, in its acute forms, causes inflammation and death of motor neurons of the spinal cord and brainstem, leading to paralysis, muscular atrophy, and often deformity- poliomyelitis sequelae (PS). Post-poliomyelitis syndrome (PPS) is a clinical syndrome of new weakness, fatigue and pain, which can occur several decades following recovery from paralytic poliomyelitis. Fatigue is defined as a state with reduced capacity for work following a period of mental or physical activity. One of the main symptoms of these patients is fatigue.

Objective: To study fatigue in polio survivors and to compare fatigue severity in patients with PS and patients with PPS.

Methods: We performed a retrospective analysis of medical archives of 303 outpatients with prior polio

paralytic history, followed at the neuromuscular sector of UNIFESP/EPM that answered Fatigue Severity Scale (FSS) questionnaire in the first evaluation. We compared patients with PS and PPS using χ^2 analysis with significance level ($p \leqslant 0.05$) and OR value with 95% confidence interval.

Results: The whole group was composed of 181 women and 122 men, mean age 46 ($25-78 \pm 9.5$) years. Eighty-eight (29.0%) patients had PS and 215 (71.0%) had PPS. Non-fatigue was found in 52 (53.1%) in PS and 46.9% in PPS group. Fatigue severity was worse in the post-polio syndrome: mild fatigue was observed in 16 (34.8%) patients with PS and 30 (65.2%) with PPS. Moderate fatigue was present in 10 (18.9%) patients with PS and 43 (81.1%) with PPS. Severe fatigue was found in 10 (9.4%) patients with PS and 96 (90.6%) patients with PPS. There was a significant statistical difference between groups in the severity fatigue variables.

Conclusion: Fatigue is presenting in both groups; however, fatigue is high more frequently and most severe in the post-poliomyelitis syndrome than in the poliomyelitis sequelae group.

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P11 COMMUNICATION METHODS FOR ALS PATIENTS USING A TELEVISED MOBILE PHONE SYSTEM

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Background: ALS is a progressive neurodegenerative disorder, and dysarthria worsens gradually. The patients in advanced stages of this disease are not able to join in social activities or enjoy self-supporting life.

Objective: To determine the utility of a televised mobile phone system as a means of communication support for ALS patients.

Methods: A 48-year-old male ALS patient living in Chiba City cooperated in the study after informed consent. He had been living with the disease for six years and wearing a mechanical ventilator for four years.

Stage 1. Preliminary experiments were performed on telecommunications using a televised mobile phone system between the Study Group Secretariat in Miyagi National Hospital and the patient's room in Chiba City with technical aids provided by a visiting technician.

Stage 2. In an educational session for patients with intractable disease, sponsored by the Miyagi Council for Intractable Disease Care, the patient provided a remote lecture on peer support from his room to the venue via the televised mobile phone system.

Results: 1) Preliminary experiments: the telecommunications between the Study Group Secretariat and the patient's room were successful with good quality in both sound and images. 2) A total of 90 people participated in the session, including patients' families and medical care personnel. The lecture with the theme 'Peer Support – Empower Our Fellows through Creation' took place using the televised mobile phone system, featuring the patient's images from his room in Chiba City and voices generated by a computerized communicator into which he input sentences by himself. The participants at the venue in Sendai City were able to listen to the lecture while watching the lecturer being projected on a screen. However, the voices were much less audible, owing to sound distortion, compared to the preliminary experiments when the volume on the speaker system was maximized. This was possibly because of the spatial situation at the venue where the system was used and the system's acoustic performance (i.e. personal computer, mobile phones, and speakers).

The session was highly rated by the participants. Their opinions included ''I was deeply impressed by the patient's first-hand talk and I highly appreciated the use of the TV phone system'', and ''The use of the latest equipment by patients will extend the range of their daily activities and lead to their self-fulfilment''. These opinions suggest the possibly increased feasibility of care support, including peer support, provided by a patient for other patients, using the televised mobile phone system.

Conclusion: In recent years there have been increasing opportunities for ALS patients wearing a mechanical ventilator to go outside their home, which imposes significant financial burdens associated with caregiver allowances, transportation fees and other expenses. In the present study, the use of the televised mobile phone system enabled the patient to make the remote lecture without the aid of caregivers. This system will enable patients with intractable disease to join social activities and may help them enjoy self-supporting life.

P12 PERSONAL COMPUTER COMMUNICATION RESTORATION SOLUTIONS FOR THOSE WITH ALS/MND

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Background: Taiwan is estimated to have between 800 and 1000 people with ALS/MND, but the true figure remains unknown. Of the around 250 registered with the Taiwan MND Association, over half have been identified as requiring AAC support. However, only a minimal number have actually received a communication aid, something the Association urgently seeks to redress. As standard AAC strategies will take time to reach these shores, and with limited government funding, other solutions are needed. This report should be read from a

perspective of a country with developing rehabilitation social service structures.

Objectives: To find a low cost but effective way to resolve the communication difficulties of those with ALS/MND in the Taiwan regional area.

Methods: Taiwan is a computer literate society and has the benefits of low cost computer hardware. The concept was that, as many homes possess a personal computer, if this was used for communication restoration, the problems of cost and lack of AAC services could be largely overcome. However, to achieve this, alternative computer control devices have to be used, as few MND non-verbal users have the necessary motor control for mouse operation. With a suitable alternative device, users would be free to use standard computer packages, such as Word, Power Point etc. for communication purposes. The user would also be provided with full access to other computer applications, such as multimedia, web access, etc. The device assessment outcome trade-off was always between speed and reliable operability.

Results: Apart from TLS cases, successful devices could be found for all evaluated cases. Usage varied, but communication was restored to all. Although limited to only seven clients, the often dramatic increase in client quality of life has validated this method for communication restoration.

Table I.

Assessment results				
No	Case ID	Device	Usage	Still in use
1.	Jin	Mini Joystick	Light	N
2.	Guan	Multi Switch Mouse	Light	Y
3.	Yang	Mini Joystick	Light	Y
4.	Lin	Adapted Std. Mouse	Heavy	Y
5.	Cai	Single Switch Mouse	Heavy	Y
6.	Su	Head Tracker	Light	N
7.	Liu	Eye Tracker	Heavy	Y

Discussion and Conclusions: All available alternative mouse devices were offered, but due to the progressive nature of the disease, in the future, assessment strategies will focus more on reliable operability and will see more use of single switch solutions (1). Restoring full PC control rather than focusing on only AAC devices has also provided client access to the world beyond the immediate environment. For those with only eye movement, studies into an eyeball switch/single switch mouse combination are underway.

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P13 THE COMMUNICATION METHOD FOR ALS PATIENTS WITH TPPV USING A LED SENSOR TO DETECT EYE MOVEMENT FROM EARLY PHASES OF THE DISEASE

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Background: For ALS patients with relentlessly aggravating motor impairments, support for their difficulties in pushing a switch for call is indispensable. In our hospital, when the patient's finger movement was not able to grasp and push the usual nurse call, we systematically changed the call devices with various types of sensors and means for switching such as grasping, pushing and touching, depending on the residual voluntary movements of the patients. ALS patients, however, always feel anxiety that they will not be able to use the current switch in the near future. On the other hand, ocular movement is the least affected voluntary movement, but it was generally used for a switch only after any other movement switches became unusable. This is partly due to the fact that the current call devices detecting the electrical activities of the extra-ocular muscles by surface electrodes cannot necessarily pick up the patient's signs correctly.

Objectives and methods: To use the relatively well-preserved ocular movement of ALS patients as a measure of switching from the early stage of the disease, we developed a switching device using a LED sensor, which can detect the difference in the light absorbance of white and black (or brown) colour of the eyeballs. To solve the problems of adjusting the subtle position and angle of LED sensor just before the eyeball, we developed a system using goggle glasses. To evaluate the usefulness of our device, we applied it to four ALS patients and nine healthy controls with informed consent.

Results: We attached a small transparent rubber sucker to the peripheral outer surface of the goggle glasses so as not to obstruct the patient's visual field. A LED and fiber sensor with leads to a battery was installed on a piece of flexible short wire fixed in the middle of the sucker. In this way, we could change the position of the sucker with LED sensor freely on the surface of the goggle glasses, and also change the direction of the leads to any direction. The light of LED could be projected onto the desired position of the eyeball easily. We defined that if a subject looked at the LED light it meant "Yes" and if not, it meant "No".

The signals from 13 subjects using our device were stable and we could judge their signs correctly. Both ALS patients and healthy controls could look at the LED light transiently (within 1 second) or for several seconds. The latter 'long switch on state' may be applied to mean a third sign other than the Yes or No sign. The comments of ALS patients and controls were as follows: 1) This device is safe for their eyes; 2) It makes it possible to detect patient's intentions accurately; 3) It may be applied not only to

simple Yes or No signs but also to signals for other more complicated communication devices.

Discussion and conclusions: Our device using LED sensor can sensitively detect the difference in the light absorbance of colours of the eyeballs and exchange the signals to Yes or No signs correctly. Compared to previously reported LED devices, our method is safe for eyes, easy to set and has possibilities of various uses from the early stage of the disease.

P14 THE CHINESE ALS HEALTH PROFILE STUDY: PRELIMINARY REPORT OF ALS CLINIC STUDY GROUP IN CHINA

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Background: ALS starts with progressive muscle weakness, wasting and fasciculations, in conjunction with spasticity, hyperreflexia and pathological corticospinal tract findings characterized neuropathologically by degeneration of motor neurons in the brainstem, spinal cord and cerebral cortex. Clinical syndrome evolves to death in 3.5 years. Substantial variations exist in managing ALS in China, but there has been no database to measure ALS patients' health profile.

Objectives: The Chinese ALS Health Profile Study (HPS) was designed as a longitudinal survey of patients diagnosed with ALS. It was created to improve the quality of care for patients with ALS by 1) obtaining the profile of the patients diagnosed in China, 2) obtaining data on the physicians-perceived health status and well-being for patients with ALS, and 3) surveying the difficulties of the patients in complying with the treatment on a long-term basis.

Methods: This observational database is open to six ALS clinics in three cities (Beijing, Shanghai, Guanzhou) of China. Data were collected between January and June 2002 by using standard data collection instruments. Forms were submitted to a central data-coordinating centre. The Chinese ALS-HPS has a First Visiting Form and Follow-up Form which include general information, physician assessments and therapeutic interventions or aids currently in use. General information comprises name, sex, birth date, height, weight, hospital of diagnosis, month and year of symptom onset and diagnosis. Physician assessments comprise region affected, ALS type (familiar/sporadic), diagnostic classification, diagnostic test performed (e.g. EMG, motor NCV, conduction block test, routine blood test, thyroid function, serum anti-GM1 antibody, brain/spine MRI, muscle biopsy) atypical features, forced vital capacity, ALS Functional Rating Scale, and SF-36. Current clinical problems and medications were also recorded.

Results: Beginning in January 2002 through to 30 June 2002, 139 patients were enrolled at six clinical sites in three cities of China. On enrolment, patients had a mean age of 49.17 years, 68% were men and 88% were sporadic. The mean time from first symptom onset to confirmation of diagnosis was about 18 months. EMG was performed in 94% of patients. Brain and spine MRI, blood and CSF screening were widely used. Seventeen per cent of patients experienced depression. Riluzole was prescribed for 19% of patients overall. Vitamins or antioxidants and Chinese traditional medicine was prescribed for 45% and 25%, respectively. Physical therapy was the most common non-pharmacological intervention (13.67%).

Conclusions: The Chinese ALS HPS appears to provide valuable data on physician practices and patient health profile in ALS. The information generated will help to identify problematic areas of management, which can be used to help to issue practice guidelines for ALS management in China, and early diagnosis should be emphasized.

P15 INDIVIDUALIZED QUALITY-OF-LIFE MEASUREMENT FOR PATIENTS WITH NEUROLOGICAL 'INTRACTABLE DISEASE' IN JAPAN

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Background: Conventional quality of life (QoL) scales often have questions that are not relevant to many patients. The direct-weighting version of the schedule for evaluation of individual quality of life (SEIQoL-DW) can give more appropriate indicators of QoL.

Objectives: To develop a version of the SEIQoL-DW for use in Japan, and to promote its use by health professionals caring for patients with neurological 'Nanbyo' ('intractable diseases').

Methods: In 2000 we used a preliminary Japanese version of the SEIQoL-DW in the introductory part of a qualitative study of ALS patients and caregivers. In 2003 we had a special training program on the SEIQoL-DW by two major government-supported groups carrying out research on neurological intractable diseases. In that program we collected comments by participants. Considering their feedback, we improved the Japanese version and a back-translation was reviewed by one of the original authors in 2005. We surveyed the state of utilization of the SEIQoL-DW by sending e-mail questionnaires to 49 registered users.

Results: *Statistic*s. Responses (one per institution), 20; Number of cases of administration (per researcher), 0–50; Diseases – ALS, 8 researchers, stroke, 3, respiratory disease, 1; Average time spent for one exam, 20–40 minutes.

Reasons for using the SEIQoL-DW. Closer to real QoL; can follow QoL even when patients' hopes and needs change; useful for evaluation of palliative care by health professionals; can reflect subjective QoL and is useful therapeutically.

Problems in practice. Can be invasive and harmful if examiner is not well trained; patients sometimes have trouble understanding concepts of 'levels' and 'weighting'; time-consuming; number of cues is sometimes less than 5; difficult to use with elderly patients; direct-weighting instrument is difficult to handle (the instrument has five interlocking disks, which form a type of pie chart and can be rotated to indicate the relative importance that the patient assigns to each of five different areas of life).

Discussion and conclusions: The SEIQoL-DW is an excellent tool to facilitate communication between patients and health professionals, and is a unique indicator of QoL for clinical research. We will continue promoting its use. We will also evaluate the psychometric properties of the Japanese version of the SEIQoL-DW, and we will consider easier methods of administration (computer-based).

P16 IDENTIFYING CARE ELEMENTS FOR PALS PROVIDED BY PERSONAL ASSISTANTS

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Background: PALS in Japan have been provided with government funded home care services since 2000. Non-professional personal assistants without any medical qualification are therefore urgently needed to provide home care services, which include tracheotomy suctioning at a patient's home. With that, there arises a need of identifying a standard of care among these unskilled caregivers.

Objective: We aimed to identify the standard daily care for PALS provided by personal assistants.

Method: We observed four PALS who had been receiving HMV for more than one year at their homes in Tokyo. A researcher recorded the frequency and situations of each care service or needs of the PALS on a minute basis throughout a day. Care service included respiratory management. When there were more than two assistants caring for a PALS at the same time, the total amount of care and time was calculated. The observations were recorded on a standard form. We then sorted the data into professional work and non-professional work.

Results: The total time of respiratory management for one male PALS was 55 minutes a day. Three female PALS needed tracheotomy suctioning once or twice an hour, excluding sleeping hours; the total time ranged from 177 to 200 minutes a day. In addition, 'to keep a close watch' was the most needed care, which required a total time of 171 to 527 minutes a day. 'To keep a close watch' was to closely observe or monitor signs and symptoms of pain or uncomfortable feelings, general conditions, communication need, and airway management (tracheotomy suctioning).

Although personal assistants can provide some medical care including tracheotomy suctioning, because of a lack of proper training they still need support, advice or consultation from medical professionals at the care receiver's home at any time.

Conclusion: Low cost long-term home care can be successfully achieved by utilizing care services of personal assistants who have received proper training from medical professionals.

P17 THE ALS-SPECIFIC ADVANCE DIRECTIVES

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Background: Patients with ALS encounter severe problems with nutritional and respiratory support when the disease progresses. The predictability of the natural history allows them to face the process of planning for their future medical care. Thus, advance directives might be a useful tool for improvement of their quality of life.

Objectives: The purpose of this study was to develop the ALS-specific advance directives.

Methods: Patients with ALS at Seirei Hamamatsu General Hospital were recruited for the study. Retrospective data about advance directives were collected from hospital records between 1995 and 2004. According to the obtained findings, a list of terms and definitions that affected clear decision-making was explored to develop the ALS-specific advance directives. Finally, the developed format for advance directive was applied to patients newly diagnosed with ALS since 2004.

Results: Eighty-nine patients were recruited. Prognosis and treatment options of ALS were openly discussed with 77 patients (87%), of whom 7 patients (9%) chose respiratory support, 24% nutritional support without ventilation, and 31% neither support. These decisions were described without the uniform format, and hospital records provided insufficient information about the reason for their choices. Moreover, the remaining patients' decisions could not be

detected in hospital records. These findings generated the uniform format for advance directives, which consisted of understanding of the informed consent and definitions of future medical care. Additionally, a feature of this format was that the patient could reject the immediate decision, while he/she should describe the reasons of his/her decision regardless of the choice. Upon adequate explanation of ALS (1), the novel format was performed without any trouble in eight patients.

Conclusions: The developed format for advance directives in the study is anticipated to be ALS disease-specific and to be useful in approaching conversations with patients about the end of life.

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P18 END OF LIFE DECISION MAKING: A CROSS CULTURAL STUDY

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Background: The care of the person with MND/ALS is often complex and, as the disease progresses, there are many decisions that need to be taken. These are even more prominent as the later stages are reached, as decisions about the care at the end of life are necessary in anticipation of the deterioration, which may be only over a few days.

Objectives: The aim of this study is to look at the different approaches to end of life care in three countries – the UK, USA and Japan.

Methods: A questionnaire has been developed to allow the retrospective audit of the notes of patients who have died under the services.

Results: In the UK 47 sets of notes have been analysed and have shown that there are discussions with patients on some issues – in particular gastrostomy insertion – in 77% of cases, with a mean time before death of 9.6 months. Discussion on advance directives occurred in only 30% of cases, with a mean time before death of nine months, and for DNAR (Do Not Attempt Resuscitation) in 36% with a mean time before death of 6.1 months.

The analysis of 10 notes from the USA showed that advance directives were discussed with 80% of patients; DNAR was discussed with 70% and both were discussed with a mean of 10 months before death.

Fifty-five notes from eight units in Japan were analysed; advance directives and DNAR had been discussed with 60% of the patients, although the details of decisions regarding end of life were unclear. However, the decisions made were different as 36% of patients received non-invasive ventilation for a mean time of five months (UK 15% for a mean of 11 months and USA 20% for a mean of five months) and 11% received invasive ventilation for a mean of 6.7 years (UK two patients for a mean time of eight months, USA none).

Discussion: These results show that there are differences in the discussion and consideration of end of life issues within different units and countries. Decision making appears to vary and in particular the use of ventilatory support is used more often in Japan than in either the UK or USA.

As the care of patients develops, and the use of international guidelines continues, these areas will need to be considered further. The discussion regarding end of life will become increasingly important, particularly as there is now awareness of cognitive impairment, and possible reduction in decision making capability. If professionals are to be able to undertake the wishes of their patients, and families, these discussions are necessary earlier in the disease progression, while decision making is possible.

P19 PREFERENCE-BASED QUALITY-OF-LIFE MEASURES IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Preference-based measures are a type of health-related quality-of-life (QOL) scale designed to measure the relative utility value, which can be used to calculate quality-adjusted life-years for cost effectiveness analysis. Significant differences were previously reported among three preference-based scales in a sample of patients with Parkinson's disease (1). It is uncertain that preference-based scales are appropriate for patients with neurodegenerative/neuromuscular diseases.

Objectives: To assess the validity of using preference-based scales in patients with amyotrophic lateral sclerosis (ALS).

Methods: Twelve patients with ALS were rated using two preference-based scales, EQ-5D (2) and HUI (3). At the same time, we measured ALSAQ-40 (4,5) and scales of clinical severity and activities of daily living (ADL), ALSFRS-R (6), Norris scale, Appel scale (7), Barthel index and motor function items of Functional Independence Measure (FIM-M).

Results: All the clinical and ADL measures were highly correlated with one another. Scores from the EQ-5D and

HUI correlated well with the ALSAQ-40 and all the clinical and ADL measures. The value for the EQ-5D was highly correlated with the HUI ($p=0.003$ for Spearman's rank-order correlation). The mean scores were different; EQ-5D was 0.411 ± 0.566, the HUI was 0.13 ± 0.58.

Discussion and conclusions: In the patients with ALS, the EQ-5D and the HUI correlated well with measures of clinical severity and ADL, and each other. However, they gave strikingly different values. This difference may result in the discrepancies in cost-effectiveness analysis with ALS. Progression of the disease might affect the relative utility value measured with preference-based QOL scales. These results suggest that care should be taken when applying preference-based QOL measures to ALS.

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THEME 2 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P20 ASSESSMENT OF HYPOVENTILATION IN AMYOTROPHIC LATERAL SCLEROSIS: MULTIMETHODOLOGICAL SIMULTANEOUS MEASUREMENT

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Background: Pulmonary dysfunction resulting from weakness of the respiratory muscles is critical for patients with amyotrophic lateral sclerosis (ALS). Assessment of hypoventilation is important for medical management of ALS (1). Several methods for assessment of pulmonary function have been reported along with their advantages and disadvantages (2).

Objectives: To determine the correlation among various tests for ventilatory function in ALS.

Methods: We evaluated ventilatory function in 10 patients with ALS using spirometry, analysis of arterial blood gas (ABG) and nocturnal pulse oximetry (NPO). We measured VC, %VC, FVC, %FVC and peak flow with spirometry; $PaCO_2$, PaO_2 and pH with the analysis of ABG; mean and minimum value of SaO_2 and mean pulse rate (PR) with NPO. We defined a summary index of NPO in an earlier report (3) as follows: nocturnal hypoxia index (NHI) = (% time of $95\% \geqslant SaO_2 > 90\%$) + (% time of $90\% \geqslant SaO_2 > 85\%$) × 2 + (% time of $85\% \geqslant SaO_2 > 80\%$) × 3 + … To analyse the correlation, we calculated Spearman's rank correlation coefficient among measured values.

Results: In one patient, NPO showed periodic hypoxemia, compatible with hypoventilation in REM sleep reported in ALS (4) and Duchenne muscular dystrophy (5). Significant correlation ($p < 0.05$) was found between: $PaCO_2$ and NHI; $PaCO_2$/minimum SaO_2 in NPO; and all values measured in spirometry. Scattergram showed that %FVC decreased earlier than elevation of $PaCO_2$ and NHI.

Discussion and conclusions: Statistical analysis suggested different meanings of values in NPO. Analysis of ABG and NPO may be useful for evaluation of hypoventilation in advanced ALS with difficulties for spirometry. The relationship between $PaCO_2$ and NPO was still ambiguous. Longitudinal study might make it clear.

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P21 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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P22 USEFULNESS OF SNIFF NASAL INSPIRATORY PRESSURE (SNIP) FOR JAPANESE PATIENTS WITH ALS

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Background: As an assessment tool for respiratory function of patients with ALS, forced vital capacity (%FVC) is thought to be standard. However, it is sometimes difficult to measure %FVC in advanced stages of disease. Therefore, SNIP has been reported as a new assessment tool for respiratory function (1). However, SNIP data measured in Japanese patients are insufficient, and usefulness of SNIP is not clear yet in Japan.

Object: The purpose of this study was to investigate usefulness of SNIP in Japan.

Methods: Twenty-five healthy controls (21–57 years of age), and 30 patients with ALS (41–79 years, disease duration 0.3–13 years) were enrolled in this study. As outcome measures, SNIP, maximal mouth inspiratory pressure (PImax) (healthy control only), %FVC and $PaCO_2$ (ALS only) were collected.

Results: An average value of SNIP was 79.4 ± 21.8 cm H_2O in healthy men, 58.2 ± 17.8 cm H_2O in healthy women, 30.3 ± 20.1 cm H_2O in patients with ALS. High correlation was observed between SNIP and Pimax ($r=0.61$), %FVC ($r=0.53$) and $PaCO_2$ ($r=-0.49$), respectively. In addition, SNIP and $PaCO_2$ were correlated in ALS patients in whom measurement of %FVC was technically impossible ($r=-0.61$). A weak correlation was observed between SNIP and $PaCO_2$ in patients with bulbar symptoms ($r=-0.35$).

Discussion and conclusions: Because SNIP values in healthy Japanese people and patients with ALS were lower than in Europeans and Americans (1,2) it is necessary that original standard values are established in Japan. Since correlation was observed between SNIP and Pimax, %FVC and $PaCO_2$, it was confirmed that SNIP was reliable also as an assessment of respiratory function in Japanese people. It was suggested that SNIP was especially useful as a predictor of hypercapnia. In addition, SNIP can be measured in patients in whom measurement of %FVC was technically impossible. In patients with bulbar symptoms, although SNIP tended to be lower than in patients without bulbar symptoms, SNIP and $PaCO_2$ were weakly correlated.

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P23 RESPIRATORY MUSCLE STRENGTH AND COUGH CAPACITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Ineffective cough is a serious problem in patients with amyotrophic lateral sclerosis (ALS). This makes the patients highly susceptible to potentially fatal respiratory infections.

Objectives: The purpose of this study was to investigate the relationship of voluntary cough capacity and respiratory muscle strength.

Methods: Twenty-seven patients with ALS were investigated. The vital capacity (VC) in sitting and supine position, maximum inspiratory (MIP) and maximum expiratory pressure (MEP), and unassisted peak cough flow (PCF) were measured.

Results: The mean VC in sitting and supine positions was 35.09% (1330.00 ml) and 27.90% (1051.85 ml) of normal predicted value, respectively. VC in the sitting position was of significantly higher value than in the supine position. The mean PCF was 205.37 l/min. The mean MIP and MEP were 26.48% (21.48 cm H_2O) and 27.96% (33.41 cm H_2O) of normal predicted value, respectively. Both MIP and MEP were correlated with PCF.

Conclusions: For assisting cough effectively, it is important to analyse the correlation between cough capacity and the strength of respiratory muscles. Both MIP and MEP,

which are the markers of respiratory muscle strength, should be taken into account in the study of cough effectiveness.

P24 RELATIONSHIP BETWEEN TIME FROM ONSET OF ALS SYMPTOMS AND INITIATION OF HOME MECHANICAL VENTILATION (HMV) IN BULBAR AND SPINAL ONSET ALS PATIENTS AND DIFFERENCES IN ADVANCE DIRECTIVES CONCERNING INVASIVE MECHANICAL VENTILATION (IMV)

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Background: ALS is a progressively paralysing disease resulting in poor quality of life and death due to respiratory failure. HMV has been shown to improve quality of life and survival. The degree of progression in the loss of respiratory function has been shown to be related to the onset of symptoms, with a faster decrease in bulbar onset compared with spinal onset patients. These factors may have an impact on the time of initiation of ventilatory intervention and on the patient's ability to make an advance directive concerning IMV.

Objective: To determine the influence of bulbar and spinal onset in patients with ALS on the timing of HMV, and on the ability to make an advance directive concerning IMV.

Methods: Data were collected retrospectively from ALS patients cared for at our centre in 2005 and for whom HMV, non-invasively (NIV) and invasively had been ordered. The patients had been diagnosed with ALS/MND by El Escorial revised criteria, and were referred for respiratory evaluation when patients presented symptoms of nocturnal hypoventilation and/or forced vital capacity (FVC) decreased to 70% of predicted. Mechanical ventilation was initiated after episodes of acute respiratory failure or after evaluation of polysomnographic studies. All patients were informed of the possible respiratory complications and the therapeutic options in an attempt to achieve early decision-making. Onset of symptoms and time from start of symptoms to initiation of mechanical ventilation was recorded. Furthermore, onset of symptoms and time from start of symptoms to decision to make an advanced directive was recorded in the NIV group.

Results: Fifty-two patients were identified, 27 (52%) with bulbar onset and 25 (48%) with spinal onset. Thirty-eight were ventilated non-invasively and 14 invasively.

The mean time from start of symptoms to initiation of HMV was, in the bulbar onset group, 23 months (range 10–60 months) and in the spinal group 34 months (range 9–84 months) ($p < 0.01$).

In the bulbar group, 88% made an advance directive after a mean period of 30.7 (range 11–72) months, where 40% accepted and 48% refused IMV; 12% did not make an advance directive at all. In the spinal group, 66% made an advance directive after a mean period of 44.1 (range 11–72) months, where 38% accepted and 29% refused IMV; 33% did not make an advance directive at all.

Conclusions: In our population of ALS patients the onset of symptoms had a significant impact on the timing of initiation of HMV with the shortest time in the bulbar onset group. This knowledge may imply a more aggressive approach concerning respiratory monitoring and information, and in terms of advanced directives an early attempt in giving information to approach early decision-making in this subgroup of patients. For the spinal group a large number of patients did not make an advance directive at all. This may imply a more careful practice in giving information to approach decision- making for all patients in this subgroup.

P25 DIFFICULTIES, PSYCHOLOGICAL SUPPORT, AND RELATIONSHIP WITH HOPE IN INVASIVE MECHANICAL VENTILATOR-DEPENDENT ALS PATIENTS IN JAPAN

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Background: Most ALS patients who depend on an invasive mechanical ventilator (IMV) suffer from movement, speech, and swallowing deficits, while at the same time facing nearly insurmountable barriers in communication of their suffering. While it is crucial for such patients to depend on psychological support for a sense of hope, no research on these factors in IMV ALS patients has yet been performed.

Objectives: This research seeks to provide a basis for improving IMV ALS patient care by investigating their difficulties, sources of psychological support, their joys and happiness, and their desires and wishes. The relationships between these factors and patient attributes are explored, followed by assessment of relationship to hope as an indicator of successful adaptation to the illness experience (assessed by the Herth Hope Index).

Methods: We first performed interview surveys, then used the results from these to craft postal surveys using anonymous questionnaires. We interviewed 27 patients and their families, and obtained valid responses from 157 subjects for the written survey (recovery rate of 78.8%).

Results: Most patients suffered multiple kinds of physical, emotional and social difficulties, which were correlated with lower levels of hope. Patients with more severe physical symptoms also suffered from more types of difficulties. Ninety per cent of patients said that a particular person provided them with psychological support or some source of joy and happiness. Only 30% of patients claiming someone providing psychological

support reported help from 'friends or acquaintances' and other 'supporters', while 60% of those claiming a source of joy and happiness described 'learning or education' and 'activities'. Patients describing more categories had higher levels of hope. All patients described desires and wishes in some form, with health of family members ranking the highest. Patients using computer-assisted communications tended to have more categories of people providing psychological support, joys and happiness, and desires and wishes.

Discussion and conclusions: These results show that care should focus both on alleviating difficulties and on providing effective psychological support. This double-pronged approach has the potential to help patients maintain or recover a life-sustaining sense of hope. Furthermore, improving support systems for families who care for IMV ALS people will help not only the families themselves, but also the patients who perceive themselves as burdens.

P26 SURVIVAL OF ALS PATIENTS ON HOME MECHANICAL VENTILATION

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Background: Several studies have provided convincing evidence that non-invasive ventilation can prolong survival in patients with ALS.

Objectives: We wished to present survival data in ALS patients on home mechanical ventilation (HMV) and compare the probability of survival with other patients on HMV.

Methods: From the nationwide Swedish Home Mechanical Ventilation Register we have prospective data on patients on HMV over nearly 10 years. The patients represent a broad diagnostic spectrum that enables us to compare data in a large number of patients. Also, we present two cases of patients on HMV who have survived more than seven years after disease onset.

Results: The survival of 1474 patients on HMV is displayed; ALS patients make up 148 (10%) of these cases. The survival after starting ventilation in the ALS group was significantly poorer compared to all other groups ($p < 0.001$). ALS survival rates were 45% at 1 year, 19% at 2 years and 6% at 5 years. In contrast to what was seen in other groups, no specific patient-related or healthcare system-related factors contributed to a greater relative risk for death among ALS patients. The predominant cause of death was respiratory failure.

Discussion and conclusions: In ALS, previous studies have stated varying survival rates. In an early series of 101 patients, survival rates were 69% at two years and 33% at five years. These are considerably higher rates compared with more recent smaller studies and our report. Part of an explanation may be that all patients were ventilated via a tracheostomy, whereas this only applied to 5% of our ALS patients (at therapy launch). Trach-ventilation *per se* may be easier to carry out in patients with severe paralyses, especially of bulbar nature. We also speculate that caregivers and relatives may be more reluctant to withdraw tracheostomy ventilation than non-invasive ventilation. In another study including 122 patients, 38 used NIV more than four hours per day and had a survival rate after two years just below 20%, which is very close to the findings in our study. In a control group consisting of 52 patients who renounced ventilatory support, none were alive after two years.

In conclusion, even if HMV probably improves life expectancy in MND/ALS, survival is significantly poorer compared to all other patient groups on HMV.

P27 MANAGEMENT OF MND/ALS PATIENTS IN A SPECIALIZED RESPIRATORY CENTRE AND IN THE PRIMARY HEALTH SECTOR

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Background: The number of ALS patients on respiratory support has been growing considerably during the last decade. After launch of non-invasive or tracheostomy ventilation, the majority of the patients are discharged to their home. In some cases this is, however, not feasible. Care of ALS patients on long-term mechanical ventilation in a hospital is for several reasons not an appropriate arrangement. Residence in a ventilator-specialized institution appears to be a more satisfactory solution.

Objectives: We wish to describe the organizing initiatives and the step-wise intensification in treatment leading to home mechanical ventilation (HMV). Furthermore, we wish to relate the collaboration with a specialized nursing home for long-term mechanical ventilated patients.

Methods: We conducted a survey of the management of respiratory services in ALS in eastern Denmark covering a population of 2.7 million.

Results: Based on intermittent interdisciplinary meetings with local ALS teams, patients are referred to Respiratory Centre East. In recent years we have in this way received about 35 ALS patients annually. Initially all patients embark on intermittent day-time continuous positive airway pressure (CPAP) and if the vital capacity is below 50% of expected, nocturnal cardio-respiratory monitoring is performed. The ALS patients are encouraged to try nocturnal non-invasive ventilation (NIV) when respiratory failure is imminent. Transition to tracheostomy ventilation when patients are ventilated more than 12–14 hours per day has become more common.

Discussion and conclusions: The social welfare system in Denmark differs from that in most other countries

because almost all social benefits are free, as everything is covered via income tax. ALS patients have different options for assistance: aids and appliances, personal helpers, speech therapy and physiotherapy. Respite care or moving to a rest home, nursing home or hospice is another option.

If the patient has embarked on non-invasive or tracheostomy ventilation the Institution for Respirator Patients (IRP) may be an option. The residence may be permanent or temporary until e.g. a team of personal helpers has been trained. The ALS patients in IRP are most often patients with a permanent residence.

In conclusion, respiratory services in ALS are challenging and may demand considerable resources. IRP in many cases relieves the pressure on more expensive beds in the hospitals and offers the patient an existence under homelike conditions.

P27A VARIABILITY IN RESPIRATORY PHYSICAL THERAPY IN ALS PATIENTS

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Background: Impairments related to ALS are defined by loss of strength, respiratory insufficiency, spasticity, loss of fine motor coordination, and speech and swallowing difficulties. Physical therapy can potentially maximize muscle and respiratory function prolonging survival, independence in ADL and quality of life. This study is part of a larger study of ''Clinical Practice Guidelines based Evidence for patients with ALS/MND''.

Objectives: The purpose of the study was to analyze variability in respiratory physical therapy based on specific respiratory muscle training in ALS patients.

Methods: *Study population*. The inclusion criterion was: Physiotherapist (PT) with clinical experience in ALS patients. As heterogeneous a group as possible was formed, by taking urbanization, practice type, gender and age into account.

Qualitative Research. A telephone survey was used. Two trained interviewers conducted the semi-structured interviews using open-ended questions. An interview guide was used, which was developed and adapted through pilot interviews. The interviews focused on the type of specific respiratory muscle training given to ALS patients, frequency, time of session, treatments modalities (home exercise and supervised treatment). For example, questions from the PT interview guide were: what type of treatment do you give to ALS patients? Do you include specific respiratory muscle training at home? Do you use techniques of manual therapy?

Results: For this study, seven PT were interviewed (4 males and 3 females), mean age 28 (SD:4) years and four years of clinical experience in ALS patients. 85.7% always performed respiratory physical therapy in ALS patients. Of these, all physiotherapists carried out a supervised treatment and 71.4% also included a domiciliary treatment (home exercise).

In the supervised treatment, mean time dedicated to specific respiratory muscle was 19 minutes (mode 10 minutes). Thirty three percent performed the treatment three times a week and 66.6% twice.

In the home exercise programme, all of the physiotherapists recommended that respiratory exercise (expiratory and inspiratory) should be performed seven times a week, nevertheless a large variation exists as far as the number of times of day (14.3% recommend once; 28.6% twice and 57% three times) and mean time dedicated to specific respiratory muscle (mean 14 minutes; range 7–20 minutes).

Conclusions:

- 85.7% of physiotherapists performed specific respiratory muscle training in ALS patients but there was a large variation in the different parameters used.
- It is necessary to include the parameters of the treatment of respiratory physical therapy in the Clinical Practice Guidelines.

P27B HOW IS RESPIRATORY PHYSICAL THERAPY TREATMENT USED IN ALS PATIENTS?

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Background: In a changing health care world with increasing emphasis on patient participation and shared decision making, patients' beliefs and ideas strongly influence the process and outcomes of treatments.

ALS patients' experiences may provide unexpected insights and may help in the understanding of clinical decision making and the various alternative and complementary treatment methods.

Objectives: The purpose of this study was to identify the respiratory physical therapy care, from point of view of ALS patients.

Methods: *Study population.* The inclusion criterion was: Diagnosis of probable or definite ALS (El Escorial diagnostic criteria) in initial phase. ALS patients from Hospital Carlos III Madrid were included in the study.

Qualitative Research. A telephone survey was used. Two trained interviewers conducted the semi-structured interviews using open-ended questions. An interview guide was used, which was developed and adapted through pilot interviews. The interviews focused on type of specific respiratory muscle training in ALS patients, frequency,

time of session, treatments modalities (home exercise and supervised treatment). For example, questions from the patients interview guide were: Do you perform respiratory physical therapy? How is the specific respiratory muscle training? Who explained the respiratory physical?

Results: In this study, 20 patients were evaluated (15 males and 5 females). Only 40% of the patients indicated that they carried out the respiratory physical therapy programme. Of these, they received a treatment based on techniques of manual therapy and supervised exercises by a physiotherapist. They also carried out a treatment of domiciliary exercises taught by their physiotherapist but they did not know exactly how to perform them. Sixty percent didn't even know the time of the treatment session supervised by a physiotherapist. The treatment of this type is once a week in 50% of the cases.

Sixty percent didn't know or didn't remember the length of time they they should carry out the programme of domiciliary exercises. They didn't know or didn't remember how many days a week they should be carried out or how many times a day. They indicated that they carried out inspiratory (40%) and expiratory (30%) exercises and exercises of upper limb (16.7%). Fifteen percent of people indicated that the exercises have not been modified since the beginning and that they get bored.

Conclusions:

- The supervision of a physiotherapist is necessary to guarantee that the patient carries out the respiratory physical therapy and home exercise.
- ALS patients do not carry out physiotherapy specific respiratory muscle training correctly. They do not know or do not remember the parameters.

P28 THE SIGNIFICANCE OF RESPIRATORY PHYSICAL THERAPY (RPT) FOR ALS PATIENTS WITH TPPV

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Background: In the course of ALS, in addition to limb muscle weakness, respiratory muscle weakness inevitably occurs, leading to ventilatory impairment.

In addition to the physical therapies for ALS patients to keep the power and the range of motion for limbs, we have performed respiratory physical therapy (RPT) for them to maintain the power and flexibility of the respiratory muscles and promote the removal of sputa even after they use tracheostomy positive pressure ventilation (TPPV). The RPT includes manual breathing assistance, the range of motion exercise of the thorax, posterior lifts, mobilization of the shoulder girdle and squeezing. To continue the RPT at home, we have taught the methods to the patient's caregivers, home helpers and visiting nurses.

Objectives: We performed this study to evaluate the significance of continuous RPT on ALS patients with TPPV.

Methods: A total of 21 patients (12 men and 9 women) with TPPV, who had taken RPT more than twice a week, were included in this study. We reviewed the medical records of the patients and also conducted a questionnaire survey of the patients and their family members.

Results: The clinical parameters of ALS patients with TPPV who took RPT were as follows: mean age, 63.2 ± 10.4 years; duration of illness, 10.6 ± 7.4 years (3–25.4 years); the duration of the use of TPPV, 5.6 ± 5.2 years (0.4–19.6 years); the treatment period of RPT after TPPV, 4.0 ± 3.1 years (0.2–11.0 years). The main performers of RPT were the visiting nurses sent from home nursing stations, followed by the physiotherapists. The patients' comments on RPT were as follows: it makes them feel comfortable, relaxed and makes it easy to remove phlegm. Eleven patients out of 14, who can be transferred to a wheel chair, went out from once a week to several times a year for a change or to attend some social activities.

Discussion and conclusions: Although we cannot show the effectiveness of RPT on ALS patients with TPPV by any clinical parameters, squeezing seems to promote the removal of sputa and respiratory muscle stretch gymnastics on chest wall mobility seems to maintain the flexibility of the chest wall even under TPPV care. In addition, RPT is a kind of relaxation method through direct touch of the patient's body, and may contribute to mental support, palliative care and the establishment of the confidential relationship between the performers and the patients. Moreover, the stabilization of mental and physical status by RPT may partly enable the patients to go out.

P29 RISK OF ASPIRATION PNEUMONIA POSED BY ORAL FOOD INTAKE FOR ALS PATIENTS WITH TRACHEOSTOMY AND ARTIFICIAL VENTILATION

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Background: Patients with ALS usually stop oral food intake and use PEG when they receive a tracheostomy, because swallowing becomes more difficult than before, and may lead to aspiration pneumonia. In addition, oral food intake may be a burden for caregivers.

Objectives: We assessed the risk of aspiration pneumonia posed by oral food intake for ALS patients with tracheostomy and artificial ventilation.

Methods: We enrolled nine hospitalized ALS patients with 24 hour artificial ventilation with tracheostomy from September 2004 to April 2006. Among these nine, three patients maintained oral intake even after tracheostomy, three stopped oral intake after tracheostomy, and the other three sometimes had oral intake only some of the time. Fever of more than 38°C and use of antibiotics due to respiratory

inflammation were considered as an event. We compared the frequency of events during periods of oral food intake with periods when oral food intake was stopped.

Results: During a period of oral intake totalling 65 months, events were observed 14 times (0.22 per month). During the period when oral intake was stopped (72 months in total), events were observed 12 times (0.16 per month).

Conclusion: Although respiratory inflammation may happen slightly more frequently, the enjoyment of taking food makes patients feel happy. Oral food intake should be considered to improve quality of life for ALS patients with tracheostomy and artificial ventilation.

P30 USEFULNESS OF THE TRACHEOESOPHAGEAL DIVERSION AND LARYNGOTRACHEAL SEPARATION FOR INTRACTABLE ASPIRATION IN ALS

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Background: Swallowing difficulties sometimes result in aspiration pneumonia, which is a major life-threatening problem for patients with ALS and those with other neurological diseases. Therefore, great effort is necessary to protect patients from aspiration pneumonia. Management of patients with aspiration requires discontinuation of oral intake. Traditional surgical management has often relied on tracheostomy. This medical management is usually effective, but requires significantly more care in controlling salivary secretion because it increases the risk of aspiration. Frequent suctioning with an aspirator by medical staff and caregivers is necessary and time-consuming. This causes fatigue among patients, caregivers, and nurses alike. In cases where there is a failure to control aspiration, further surgical procedures may be necessary, such as laryngotracheal separation (LTS), which is a modification of tracheoesophageal diversion (TED), or laryngectomy. These procedures separate the airway and digestive systems and can effectively eliminate intractable aspiration. To most neurologists, little is known about LTS/TED in detail. Very few studies have evaluated the usefulness of LTS/TED or patients' and families' satisfaction with it. Indeed, as far as we could determine, it has not been recognized as a common procedure.

Objective: We investigated changes in medical management after LTS/TED had been performed, including feeding conditions and whether or not patients, family caregivers, and medical staff are truly satisfied with the outcome.

Methods: We performed LTS/TED for intractable aspiration in seven patients with ALS and nine patients with other neurological diseases. Most of the subjects had already received a tracheostomy, and all the patients with ALS had tracheostomy positive pressure ventilation. However, they remained at risk of intractable aspiration, had experienced frequent suctioning of aspiration material, and could not eat. After LTS/TED, we evaluated the efficacy of the therapy.

Results: In all cases, LTS/TED was performed safely within three hours and without any complications. After LTS/TED, there was no aspiration of saliva in any of the patients. In most subjects the frequency of suctioning by medical staff and caregivers was much reduced. The frequency of fever/infection by aspiration was also extremely reduced. Follow-up study demonstrated that complete control over aspiration was achieved in all of the patients. Six of them were completely self-sufficient in their ability to eat. Some of the other patients were at least able to enjoy taste. Nutritional status was significantly improved. All the patients, family caregivers, and medical staff involved in this study were satisfied with the outcome.

Conclusions: These results indicate that LTS/TED is a very useful procedure in several aspects: it benefits patients who have a fear of aspiration, it reduces the burden on patients and family caregivers, it promotes their quality of life and it limits the aspiration-associated demands on medical staff.

P31 ANOTHER USEFUL TUBE FEEDING METHOD FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: INTERMITTENT ORAL CATHETERIZATION (IOC)

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Background: For tube feeding of ALS patients, we have used continuous nasal catheterization or percutaneous endoscopic gastrostomy in Japan. It has been highlighted that continuous nasal catheterization has some problems as a long-term tube feeding method (1).

On the other hand, there are many complications in the management of percutaneous endoscopic gastrostomy (2).

IOC has been used as supplemental tube feeding for dysphagic children and cerebrovascular disease patients (3). We introduced IOC for patients with amyotrophic lateral sclerosis (ALS) for long-term tube feeding.

Objective: To investigate the progress of ALS patients fed via IOC.

Methods: We introduced IOC to 17 patients with ALS (58–82 years of age). The duration between the onset of ALS and introduction of IOC was 7–59 months. Before the introduction of IOC, four patients were able to eat modified food, 13 patients found oral intake very difficult

or impossible, and three patients had not been given a diagnosis at the introduction of IOC.
At every meal time, the patient or caregiver inserted the catheter orally to the stomach and pulled out the tube after feeding.

Results: IOC was introduced before the start of ventilation in 10 patients and after the start of ventilation in seven patients. Their forced vital capacity was 30–60%.

We could introduce IOC for patients under non-invasive positive pressure ventilation or tracheostomy positive pressure ventilation. The patients continued IOC for 3–36 months. Five patients who died during follow-up had continued with IOC nutrition until the end of their life. Three patients discontinued IOC because of tracheotomy, fatigue and increase of sputum. We found no complication related to IOC.

It took less than 10 sessions of training to master the skill of IOC. Almost all the patients and families felt comfortable about IOC.

Conclusion: We could introduce IOC to patients with mild to advanced stages of dysphagia, regardless of respiratory function and whether they had been informed of a diagnosis of ALS.

IOC is one of the useful tube feeding methods for ALS patients.

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P32 THE SAFETY AND EFFICACY OF PEG TUBE INSERTION IN ALS/MND PATIENTS IN A TERTIARY INSTITUTION

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Background: Percutaneous endoscopic gastrostomy (PEG) in patients with ALS/MND has been shown to improve quality of life and stabilizes or increases weight among ALS patients. Furthermore, life expectancy is prolonged by provision of adequate hydration and nutrition in patients with progressive malnutrition secondary to dysphagia. PEG insertion can be risky, particularly in the patient with borderline respiratory function. This retrospective study reviews tube placement outcomes in 39 MND/ALS outpatients seen by a multi-disciplinary team during a six-year period from 1999 to early 2006 at a tertiary hospital in Sydney, Australia.

Objectives: To determine rate of complications associated with early insertion of PEG tubes by reviewing outcomes based on measures of respiratory function, nutritional status and a decline in functional speech and swallowing. This review will enable us to inform patients with bulbar onset ALS regarding benefits of PEG tube placement and possible complications. Furthermore, it would allow us to review our current practice of early intervention, particularly in bulbar onset of ALS/MND.

Methods: A retrospective review of all PEG tube placements in an ALS/MND outpatients' clinic between September 1999 and March 2006 was completed. Data were collected from medical records, describing patients' nutritional status, respiratory function as well as speech and swallowing function at the time of tube placement. These data were related to trends seen at the initial assessment of nutritional, respiratory and bulbar function as seen in the outpatients' ALS clinic. All tubes were placed in the tertiary hospital with support of non-invasive ventilation. Full intensive care support was used if required.

Results: There were two serious complications associated with tube placement in 39 consecutive patients, with peritonitis secondary to leakage of stomach contents at the time of insertion. Both patients survived with early laparotomy, repair of the site of leakage, and replacement of gastrostomy tube at the time of laparotomy. Five patients required the replacement of PEG tubes due to mechanical failure of the tubes. There were no deaths related to tube insertion. Rationale for early tube placement was influenced by the rate of weight loss, reduced food intake, inadequate fluid intake and excessive meal times.

Conclusions: Our data indicate that early PEG tube placement in MND/ALS patients is safe and effective. These data support early tube placement with a focus on using PEG tube for survival and eating for pleasure. PEG insertion is a safe procedure, particularly when supported with non-invasive ventilation.

P33 CHARACTERIZATION AND FOLLOW-UP OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS BY SWALLOWING SCINTIGRAPHY

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Background: Deglutition deficit is one of the most frequent and invalidating symptoms reported by amyotrophic lateral sclerosis (ALS) patients. Quantitative assessment methods for severity of oropharyngeal dysphagia and response to therapy are lacking. Oro-pharyngo-oesophageal scintigraphy (OPES) allows a functional and semi-quantitative study of different swallowing phases.

Objectives: The aim of our study was to assess swallowing in a population of ALS patients by means of OPES at baseline and during follow-up.

Methods: We evaluated 38 patients (18 women and 20 men; mean age ± SD, 64.5 ± 10.4 years) diagnosed as having definite or probable ALS according to the revised El Escorial criteria. Patients, clinically assessed through the ALS Functional Rating Scale (ALSFRS), underwent OPES with ^{99m}Tc-nanocolloid by using a liquid and a semi-solid bolus at time of diagnosis, three and six months thereafter. We analysed the following parameters: oral transit time (OTT), pharyngeal transit time (PTT), oesophageal transit time (ETT), oro-pharyngeal retention index (OPRI), oesophageal retention index (ERI), and oesophageal emptying rate (EER).

Results: OPES performed with semi-solid bolus identified a higher proportion of abnormal values for swallowing parameters than that with liquid bolus. By grouping ALS patients into classes according to their bulbar ALSFRS scores, we found significantly increased OTT ($p < 0.004$), PTT ($p < 0.02$) and OPRI ($p < 0.0003$) values in patients with more severe bulbar involvement. In the follow-up a high correlation was observed between increased OPRI values with semi-solid bolus and disease progression ($p < 0.0003$). OPES was useful in determining the exact bolus percentage inhaled into tracheo-bronchial branch.

Discussion: Our findings show that disease worsening leads to enhanced OTT, PTT and OPRI values, whereas ETT, ERI and EER values do not change significantly. Such data further support the neurogenic component of dysphagia in ALS patients who present typical derangement of swallowing oro-pharyngeal stage rather than that of the oesophageal stage.

In summary, OPES with ^{99m}Tc-nanocolloid is a simple, inexpensive, non-invasive and reliable technique that allows better clinical definiton of deglutition deficits and accurate follow-up of ALS patients, by supplying semi-quantitative data on swallowing performances and percentage of bolus inhaled.

P34 LARYNGOLOGICAL DETECTION OF PRECLINICAL BULBAR SIGNS IN PATIENTS WITH LIMB ONSET AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder of unknown aetiology. While multidisciplinary medical teams may be involved in the diagnostic process, the participation of otolaryngologists in evaluation of ALS patients has been seldom reported. We have previously stressed that hyper-/hyponasality, articulation defects, voice harshness and breathiness as well as swallowing difficulties should all be viewed as possible early signs of ALS and may allow the otolaryngologist to be the primary diagnostician.

Although the first clinical presentation of ALS is usually either limb or bulbar level only, some data suggest that degeneration of motor neurons of motor cortex, brainstem, and spinal cord may occur simultaneously in ALS individuals.We hypothesize that careful laryngological examination can reveal signs of early dysfunction of the vocal cords in ALS individuals with limb onset only, and thus may indicate the early presentation of bulbar dysfunction.

Objective: To test the hypothesis that preclinical signs of bulbar dysfunction occur in limb onset ALS individuals, via assessing vocal cord changes using fibreoscopic and/or videostroboscopic examination.

Material and methods: Thirty-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. To detect vocal cord abnormalities, the standard laryngological examination, as well as the fibreoscopic and/or videostroboscopic examination, was performed in the Department of Otolaryngology, Head and Neck Surgery of Jagiellonian University in Krakow.

Results: There were no abnormalities on the standard laryngological inspection.The fibreoscopic examination revealed some signs of the early dysfunction of vagus nerve/s in 25 out of 34 patients: slowness of one/both vocal folds, lack of complete closure during phonation, unilateral decrease of vocal fold tension with limited abduction. The videostroboscopic examination confirmed the fibreoscopic findings in limb onset ALS cases and has in addition revealed: vocal fold bowing, decreased abduction of the true vocal fold and mucus pooling in some cases. In both examinations, incomplete vocal fold closure was the most frequent observation.

Conclusions: We report dysfunction of vagus nerve/s in most limb onset ALS cases studied demonstrating a clinically 'silent' bulbar dysfunction in these patients. 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.

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THEME 3 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P35 UTILITY OF A BRIEF SCREENING PROTOCOL TO IDENTIFY COGNITIVE AND BEHAVIORIAL ABNORMALITIES IN ALS PATIENTS

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Obejctives: To evaluate the utility of a brief screening battery to be used in ALS clinics for the purpose of identifying patients with cognitive and behavioral abnormalities, issues that significantly affect clinical management and decision-making regarding long-term ventilation.

Background: ALS patients were once thought to be cognitively intact with the exception of a small number of patients with frontotemporal dementia (FTD). Substantial evidence now demonstrates that approximately half of ALS patients display a continuum of frontal lobe based cognitive and behavioral abnormalities, with some patients having mild to moderate behavioral problems that do not meet the threshold for FTD (ALS behaviorally impaired, ALSbi), others with cognitive dysfunction (ALS cognitively impaired, ALSci), and a third group that meets full criteria for FTD (ALS-FTD). Busy, interdisciplinary clinics frequently lack the resources to conduct thorough neuropsychological evaluations.

Methods: Thirty-two patients who met WFN criteria for the diagnosis of ALS underwent a 20-minute screening battery consisting of the Abrahams verbal fluency test or FAS fluency test, the Frontal Behavioral Inventory, the Neuropsychiatric Inventory Questionnaire version (NPI-Q), the CNS-LS, and the Beck Depression Inventory-II.

Results: Of 32 patients studied, 20 (63%) scored in the abnormal range on the Frontal Behavioral Inventory, as indicated by a score of above 30. Most frequently endorsed items on the FBI were irritability, inflexibility, apathy, restlessness, and logopenia. Four patients (13%) scored in the abnormal range on the NPI-Q, most frequently endorsing depression, anxiety, and night-time behaviors. Eight patients (25%) scored in the impaired range on verbal fluency, as indicated by an impaired score on the FAS or 1.5 SD below the group mean on the Abrahams Verbal Fluency Test. Five of the eight patients scoring abnormally on verbal fluency also scored abnormally on behavioral measures. Depression, pseudobulbar affect, FVC, age, and ALSFRS-R scores were not statistically correlated with behavioral measures.

Conclusions: This simple screening battery appears to be sensitive in identifying abnormalities in this population. The Frontal Behavioral Inventory is a more sensitive measure of behavioral problems than the NPI-Q. The traditional FBI cut-off may, in fact, be overly inclusive, as it identified nearly two-thirds of the sample as having behavioral problems. The NPI-Q, in contrast, identified 13% of patients as having moderate severity problems in at least two areas (>12). Verbal fluency deficits were observed in 25% of these patients, which is a rate consistent with the literature. Of these patients with verbal fluency deficits, approximately half had comorbid behavior problems, suggesting that cognitive and behavioral problems among ALS patients are not always co-occurring. Sound judgment and organizational planning skills are particularly necessary cognitive abilities to make critical decisions in long-term care for ALS patients (e.g. ventilation, PEG tube). Patients and family members may benefit from a brief assessment to identify potential difficulties in making such decisions. Counselling may also inform family members and patients about how behavioral and personality changes affect family relationships and the patient's ability to comply with treatment recommendations.

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P36 THE RELATIONSHIP BETWEEN FRONTAL BEHAVIORIAL AND COGNITIVE CHANGES IN ALS

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Background: It is estimated that 51% of amyotrophic lateral sclerosis (ALS) patients exhibit at least a mild cognitive dysfunction throughout the course of their disease, with 15% of these patients meeting criteria for frontotemporal dementia (FTD) (1). However, the frontal behavioral changes that may be associated with ALS have not been well described. Clinical observation suggests that patients with ALS tend to be particularly 'nice', and they also may exhibit behavioral changes similar to those seen in FTD patients including apathy, disinhibition and/or executive dysfunction. These changes can affect patients' daily activities and increase caregiver's burden and distress (2).

Objective: Our objective is to determine the prevalence and patterns of frontal behavioral changes and their relation to cognitive impairment in ALS patients.

Methods: In 225 patients with diagnoses of probable or definite ALS by El Escorial criteria, we administered a

comprehensive neuropsychological evaluation and the caregivers completed the Frontal Systems Behavior Scale (FrsBe)(3). The population was divided into three subgroups based on cognitive assessment outcome: cognitively intact, mild cognitive impairment and moderate to severe cognitive impairment. The behavioral areas evaluated by the FrsBe test are: apathy, disinhibition and executive dysfunction. The data were analysed by comparing the FrsBe results in the three groups using an ANOVA test.

Results: Using FrsBe cut-off scores, behavioral dysfunction was found in 24.4% of ALS patients. Caregivers reported that apathy was the most prevalent frontal behavior symptom (31%), but a high prevalence of executive dysfunction (19.6%) and disinhibition (16.9%) was also reported. ALS patients with greater cognitive impairment exhibited greater behavioral dysfunction.

Conclusions: There is a high prevalence of frontal behavioral symptoms reported in patients with ALS. Caregivers report that apathy is the most prevalent behavioral symptom in patients with ALS, but disinhibition and executive dysfunction are also present. Cognitive impairment and behavioral changes may be two manifestations of the same pathologic process in patients with ALS. Future research should investigate the degree of behavioral change demonstrated in ALS patients following diagnosis.

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P37 WHAT IS THE BEST BEDSIDE TEST TO SCREEN FOR COGNITIVE IMPAIRMENT IN ALS?

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Background: The diagnosis of cognitive impairment (CI) in ALS has relied on detailed neuropsychological assessments. 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 bedside measure can predict overall CI.

Objectives: To determine how well specific cognitive and behavioral measures predict CI in ALS.

Methods: Neuropsychological assessments were performed on 55 patients at a multidisciplinary ALS center. Patients with major depressive disorders or other neurologic conditions affecting cognition were excluded. Patients completed three to five individual measures and CI was considered present in patients with scores of 1.5 or more standard deviations below the mean on two or more measures. Caregivers completed a behavioral questionnaire (FrSBe). We used logistic regression to determine which measures correlated best with our definition of CI. Then, a classification model using ROC analyses was performed on measures correlating with CI at or below $p=0.10$ to determine which test predicts impairment with the fewest errors (greatest diagnostic accuracy) based on a specific cut-off. Stepwise backwards logistic regression was performed to find combinations of tests that improve the performance of any single measure.

Results: Forty-nine per cent of patients met criteria for CI. CI did not correlate significantly with demographic or disease-related variables (age, gender, FVC, ALS-FRS score, site of onset). Four neuropsychological tests correlated with overall CI by logistic regression (Trail Making Test, ($p=0.004$), Letter Fluency ($p=0.029$), Design Fluency ($p=0.030$) and Digit Span ($p=0.031$)). The executive dysfunction subscale of the FrSBe approached significance ($p=0.052$). Once the predictors were identified, subsequent analysis using ROC classification showed that Letter Fluency made the fewest errors in predicting CI (accuracy, 77%; specificity, 73%; sensitivity, 80%). The performance was not improved by additional information. The Trail Making Test had the second highest accuracy (accuracy 71%, specificity 89%, sensitivity 50%).

Discussion and conclusions: This is, to our knowledge, the first study to systematically assess the utility of specific bedside neuropsychological measures as a cognitive screen in ALS. Letter fluency is the best measure for predicting CI in ALS according to our model, supporting its role as a screening tool. This simple bedside test improves the prediction over the roughly 50% prior probability at baseline and alerts clinicians to probable cognitive impairment. The Trail Making test may be of value for patients who cannot complete verbal fluency measures.

P38 UTILITY OF THE FRONTAL SYSTEMS BEHAVIOR SCALE (FRSBE) IN THE ASSESSMENT OF NEURO BEHAVIORAL SYMPTOMS IN ALS

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Background: The behavioral disturbances involving personal and social conduct that are initial symptoms of frontotemporal dementia (FTD) may not be detected using a traditional neuropsychological test battery. Behavioral screening measures may be more effective in detecting these early symptoms of FTD in patients with ALS.

Objectives: To examine the utility of the Family Rating form of the FrSBe in assessing behavioral changes in patients with ALS.

Methods: Forty-five patients from the Kessenich Family MDA ALS Center at the University of Miami Miller School of Medicine ($n=20$) and the Forbes Norris ALS Research Center in San Francisco ($n=25$) participated in this study. Caregivers completed the Family Rating Form of the FrSBe providing ratings of patients' behavioral functioning (i.e. apathy, disinhibition, executive dysfunction) pre- and post-illness onset. Patients received neurological examinations including forced vital capacity testing and the ALS Functional Rating Scale (ALSFRS). All ALS patients were administered verbal fluency measures and Miami patients underwent more comprehensive neuropsychological testing.

Results: Results of paired *t*-tests indicated that caregivers reported significant changes in patients' levels of apathy from pre- to post-illness onset ($p<0.01$, Miami and $p<0.001$, San Francisco). After ALS onset, 56% of patients had clinically significant ratings on the Apathy scale ($T>65$ $\geqslant$94th percentile). Clinically significant changes were noted in disinhibition and executive dysfunction, but did not reach significance at both centers. Although behavioral ratings were not associated with most of the disease-related factors assessed, ALS patients with bulbar onset disease had significantly higher post-illness Apathy scores than did those with limb onset disease ($F=4.86$, $p<0.05$). The severity of current bulbar symptoms was not associated with post-illness Apathy scores. Behavioral ratings on the FrSBe were associated with performance on neuropsychological testing. Post-illness Apathy predicted performance on verbal fluency tests ($p<0.03$). Additional neuropsychological testing of Miami patients revealed that disinhibition was associated with performance on visual spatial tasks ($p<0.001$), and executive dysfunction was associated with short delay verbal recall ($p<0.05$).

Discussion and conclusions: The FrSBe is a useful tool for assessing behavioral change in patients with ALS. It is quickly completed by a caregiver and scored. The behavioral changes observed in this study occurred independently of mood and physical disease parameters, suggesting an underlying, organic neurodegeneration. Caregiver rating scales may provide useful information about cognitive functioning even if patients are physically unable to complete formal neuropsychological testing. Future investigations should compare the FrSBe with other measures of neurobehavioral symptoms such as the Neuropsychiatric Inventory (NPI) and Frontal Behavioural Inventory (FBI).

P39 COGNITIVE SCREENING IN AN ALS CLINIC

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Background: Frontotemporal dementia is increasingly being recognized as a component of the disease process affecting patients with amyotrophic lateral sclerosis (ALS). There is still debate about the best methods for screening ALS patients for early signs of cognitive impairment.

Objectives: To compare the results of three, simple cognitive screening tests (Verbal Fluency, Trail Making A, and Trail Making B) in consecutive ALS patients. Outcomes were correlated with ALS phenotype (limb, bulbar, mixed bulbar/limb), markers of disease progression (ALSFRS), and the Beck's Depression Rating Scale.

Methods: Forty-eight patients (22 females and 26 males) diagnosed with ALS were recruited from our multidisciplinary ALS Clinic. All tests were performed during the routine ALS clinic visit. Abnormal cognitive tests were defined as: Verbal Fluency <12 words in 60 seconds; Trail Making A >40 seconds; and Trail Making B >120 seconds. Failure to perform or complete any test was considered abnormal. Comparisons between different groups were made using Student's *t*-test.

Results: Our study group consisted of 21 limb ALS, 15 bulbar ALS, and 12 mixed bulbar/limb ALS. For the total population, the average age was 60.6 (range 32–89), and did not differ significantly across subtype. Overall Verbal Fluency was abnormal in 56.3% of the total population; Trail Making A in 54.2%; and Trail Making B in 39.6%. In patients with bulbar ALS, Verbal Fluency was abnormal in 73.3%, Trail Making A in 66.7% and Trail Making B in 66.7%. In patients with limb ALS, Verbal Fluency was abnormal in 52.4%, Trail Making A in 42.9%, Trail Making B in 23.8%. In mixed bulbar/limb ALS, Verbal Fluency was abnormal in 41.7%, Trail Making A in 58.3%, Trail Making B in 33.3%. Twenty-nine per cent of the total population had abnormal values on all three tests. By phenotype, 46.7% of bulbar ALS failed all three tests; 19.0% of limb and 25.0% of mixed limb/bulbar ALS. Using the raw scores, there were no significant differences between subtypes on the Trail Making A and Trail Making B, the ALSFRS, or Beck's Depression Scale.

Discussion: All three tests are potentially useful for screening cognitive abnormalities in ALS patients in the clinic. However, in this pilot study, we found that bulbar patients were more likely to have abnormal scores on all three tests than limb or mixed bulbar/limb patients. It is difficult to determine whether abnormal results are secondary to motor weakness or true frontal executive function impairment. In addition, full neuropsychological testing is needed to confirm dementia in patients who fail screening tests. Future studies are needed to determine which screening test is most sensitive for detecting early cognitive impairment in different ALS subtypes.

P40 IS FACE RECOGNITION SELECTIVELY IMPAIRED FOR NEGATIVE EMOTIONS IN AMYOTROPHIC LATERAL SCLEROSIS?

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Background: Extra-motor structural abnormalities seem to be an epiphenomenon in patients with amyotrophic lateral sclerosis (ALS) with no evidence of cognitive change. This raises questions about how this affects the

processing of emotional material. An impact on verbal ratings of socioemotional stimuli (more positive, lower range of arousal) (1) and memory of emotional words (2) has been shown in patients. The aim of the current study was to find out if changes are selective for the quality of emotion (positive vs. negative).

Objectives: Ability to recognize different facial expressions was investigated in ALS patients. Parameters that might influence the emotional experience of a person were measured and correlated to performance in the recognition task.

Methods: Twenty-one ALS patients with spinal onset, 13 patients with bulbar onset and 11 healthy controls were compared in the performance of the Ekman 60 Faces test in the facial expression of emotion. Influence of functional impairment of the patients, age, depression and intelligence as well as quality of life, personality and coping strategies on recognition task performance were assessed.

Results: ALS patients with spinal onset and bulbar onset showed no significant difference in emotional judgement ratings of different faces. Compared to healthy controls ALS patients presented a significant impairment in the recognition of disgust ($p=0.015$) and fear ($p=0.002$) in faces. Recognition task performance correlated with functional impairments in the patients ($p=0.02$), as well as with age ($p=0.015$). IQ had only a low impact on face recognition task performance ($p=0.049$).

Discussion and conclusion: ALS patients present significant impairments selectively for the recognition of negative facial expressions of disgust and fear. This is in contrast to other findings where no significant difference was found for the same task (2). Since task performance decreased with functional impairments, the patients in the present study might have been in a more advanced stage of the disease. There has been evidence for impairments in the limbic system in ALS patients. In a previous study a loss of volume in the amygdala in a group of 22 ALS patients (including 11 patients of the present study) had been shown. The function of the amygdala as an early alarm centre of the brain might be the key to the understanding of the differences we found in the task performance of ALS patients compared to healthy controls. Our data might be interpreted tentatively as the failure of the brain of ALS patients to reach the level of attention necessary for the complex information processing of negative facial expressions due to a missing early response of the limbic system.

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P41 DEFICIENT LEXICAL ACCESS IN ALS IS ASSOCIATED WITH POOR DIVERGENT PRODUCTION AND SOCIAL INTELLIGENCE

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Background: The occurrence of deficits in pre-frontal functions in many patients with ALS is now recognized, characterized by behavioral change, as well as diminished capacities for lexical access, abstract reasoning and verbal problem solving (1). Greater characterization of verbal problem solving deficiencies in ALS is of relevance to treatment planning, particularly in the terminal stages. Divergent production is the ability to generate an alternate response set in the process of creative problem solving. Social intelligence includes the ability to comprehend non-verbal communication as well as cause and effect relationships in the course of social interaction.

Objectives: To determine whether ALS patients with frontal dysfunction have deficiencies in divergent production and social intelligence. To evaluate the relationship between verbally mediated divergent production and social intelligence.

Methods: Word generation, a 3-minute frontal task of letter fluency, was evaluated in 110 consecutive patients seen in our multidisciplinary clinic.

A subset of seven patients with deficient letter fluency agreed to undergo more extensive evaluation to characterize their capacities for problem solving. Measures administered included Guilford Alternate Uses and Consequences, verbal measures of divergent production, Expression Grouping, a visual measure of interpretation of facial expression and body language, and Cartoon Predictions, a visual measure of cause-and-effect in social interactions. Mood was assessed by the Profile of Mood States. Spearman rho correlations evaluated the relationship between letter fluency and Guilford divergent production findings.

Results: Five patients were free of depression, while two acknowledged mild depression. All patients evidenced deficiencies for Consequences, Cartoon Predictions, and Expression Grouping. Five patients evidenced deficiencies for Alternate Uses. Alternate Uses was seen to positively correlate with both letter fluency ($r=0.90$, $p<0.037$) and Expression Grouping ($r=0.836$, $p<0.019$) findings.

Discussion and conclusions: In this pilot study of a cohort of ALS patients selected for deficient letter fluency, frontal executive deficits were also detected for divergent production and social intelligence. Executive functioning change in ALS appears to be characterized by diminished reasoning across a broad spectrum of capacities. An understanding of this in the clinical setting is vital to treatment planning, with greater emphasis needed on support of both patient and caregivers in the process of decision-making throughout the course of the disease process.

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P42 AGRAPHIA IN ALS: THE OMISSION OF KANA LETTERS

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Background: It is generally agreed that various cognitive dysfunctions can occur in classical ALS. We have already reported a patient with classical ALS who showed the omission of kana letters (OKL) in writing. Now we have performed neuropsychological investigations including writing for 13 patients with classical ALS.

Objectives: To clarify the frequency and mechanism of the OKL, the symptomatic pattern of ALS which causes the OKL, and the presence of morphological characteristics by neuroimaging studies.

Methods: Subjects were 13 patients with classical ALS (right handed, male 5, female 8, 47~79 years of age (mean 63 years of age), disease duration <1.5 year, normal respiratory function, independent daily life, characteristic change is absent. We performed physical examinations, the Western Aphasia Battery (WAB) writing test, Raven Progressive Colored Matrices (RPCM), and brain MRI. We also required subjects to write down the names of common animals and tools that were represented by line-drawn cards.

Results: In writing kana, the OKL was observed in two patients who were intellectually normal. Both patients had bulbar ALS, and revealed disturbance of resolution to 'mora', which means sub-syllabic rhythmic units of kana. In brain MRI, one patient revealed atrophy of bilateral frontal and temporal lobes; the other was normal.

Discussion and conclusions: In writing Japanese kana, three cognitive steps are hypothesized: the first is the resolution to mora. The word is analysed phonologically to a single syllabic unit. The second is phonological extraction. Each verbal sound is applied onto each syllabic unit. In addition, the third is the correspondence between the sound and kana. Each kana is chosen corresponding to each verbal sound. It was supposed that the OKL was caused by the disturbance of mora resolution. Mishima reported that the SPECT of a patient with the OKL in writing kana showed a decrease of regional cerebral blood flow (rCBF) in the bilateral frontal lobes. Ludolph pointed out that, in classical ALS, rCBF of the bilateral frontal lobes was often decreased. Thus, we may say that the OKL might have a relationship with frontal dysfunction. In conclusion, we should notice that, in bulbar type of ALS, some patients revealed the OKL in writing from the early stage of the illness.

P43 OCULAR APRAXIA AND COGNITIVE IMPAIRMENT IN ALS

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Objective: To describe concurrence of ocular motility abnormalities and cognitive impairment in ALS.

Background: A variety of ocular motor abnormalities are reported in ALS. Cognitive impairment also occurs in up to half of patients ranging from mild executive and behavioral dysfunction to FTD. The co-occurrence of these two phenomena has rarely been articulated.

Design and methods: We performed neuropsychological testing in 52 ALS patients suspected of having cognitive abnormalities and manual ocular motor examinations looking specifically for ocular apraxia (frequent saccadic intrusions and difficulty in disengagement of fixation when initiating voluntary saccades) in more than 200 patients with ALS. Video recordings were obtained to demonstrate the ocular motility difficulty.

Results: Seven patients had ocular apraxia, as defined above. The ocular motility abnormalities were most severe on vertical eye movements but were present on horizontal gaze in each case. The frequency of the saccadic intrusions could be reduced by having the patient point with a finger at the moving target during testing. Six of the seven patients had bulbar onset and one had limb onset but had developed bulbar involvement by the time the ocular motility abnormalities were discovered. Examination suggested that the bulbar weakness was primarily upper motor neuron in nature (slow, non-fasciculating tongue). All patients were cognitively impaired (defined as scoring at least 1.5 standard deviations below age-adjusted means on at least two tests in a cognitive battery). They also had profound behavioral impairments, marked primarily by the presence of apathy and executive dysfunction. Scores on the FrSBe for apathy were 4.5 standard deviations above the mean for the general population and were still 2 standard deviations above other patients with ALS who had cognitive impairment but had normal eye movements. As a group these patients had poor outcomes, with four surviving less than 15 months from the first symptom and five surviving less than six months from the time ocular apraxia was initially discovered. We did not find these ocular motor abnormalities in any cognitively-impaired patients who lacked bulbar involvement or in any non-demented ALS patients.

Conclusions and relevance: A subset of patients with ALS developed ocular apraxia. This occurs in the setting of a cognitive behavioral syndrome marked by pronounced executive dysfunction, severe apathy, upper motor neuron bulbar dysfunction, and poor outcomes. The characteristic appearance of these gaze abnormalities in patients with cognitive impairment suggests they relate to frontal lobe dysfunction, as opposed to degeneration within the motor cortex. The poor outcome for this syndrome may relate to the combined effects of bulbar dysfunction and cognitive impairment.

P44 THE DISSOCIATION OF DYSARTHRIA FROM COGNITIVE DYSFUNCTION IN ALS

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Background: The relationship between dysarthria and the presence of cognitive impairment in ALS patients has come under question. Recent studies have investigated whether cognitive dysfunction was associated with bulbar onset illness. Strong *et al.* (1) found that patients with bulbar onset disease showed greater impairment in working memory and problem-solving/cognitive flexibility and that cognitive impairment was progressive over time, but did not find a relationship between motor speech performance and cognition. Ringholz *et al.* (2) found that patients with cognitive impairment were more likely to have motor programming difficulties, dysarthria, decreased phrase length and difficulty with word finding. However, patients with bulbar onset ALS did not differ from limb onset patients in either level of impairment or pattern of cognitive performance.

Objective: To examine the relationship between cognitive performance and dysarthria in a large sample of ALS patients.

Methods: One hundred and seventy-five consecutive patients (112 male, 63 female) with a diagnosis of probable or definite ALS (El Escorial criteria) were seen for an initial neurological work-up to reveal degenerative neurological processes. Subjects completed an extensive battery of neuropsychological measures that assessed multiple areas of cognition. A motor speech examination by a speech-language pathologist was also completed.

Results: There was a significant effect of gender on cognitive performance across tests of intelligence with males performing better than females irrespective of dysarthria. There was also a significant effect of gender on severity of dysarthria with women being more dysarthric than men ($p=0.003$), probably relating to a higher rate of bulbar onset women. There was a significant overall effect of dysarthria severity on only a few cognitive test performances (Stroop test, Verbal Series Attention test, animal fluency). Post hoc analyses indicated this performance difference was in the more severely dysarthric women. There were no other significant effects of dysarthria on cognitive test performances including tests of executive skills, attention or learning and memory.

Conclusions: There does not appear to be a significant relationship between dysarthria severity and cognitive performance. There appears to be a subgroup of female patients with severe dysarthria who exhibit significantly poorer performance on some cognitive tests requiring verbal output. We conclude that dysarthria is unlikely to be related to cognitive dysfunction in patients with ALS.

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P45 EXECUTIVE FUNCTION DISORDERS AND ALS: IMPLICATIONS FOR INTERVENTION

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Background: Historically ALS has been viewed as a progressive neurodegenerative disease with a low incidence of cognitive abnormalities. Current literature indicates a significant incidence of cognitive change primarily involving executive functions (1). Executive functions are a set of processes that underlie goal directed behavior (e.g. planning and sequencing). Disorders of executive function are manifested by poor initiation, and difficulties planning and organizing strategies for problem solving. The prevalence of executive function disorders in this population prompted a change in our speech pathology intervention strategies. The typical intervention of providing patients and caregivers with lists of multiple speech and swallowing strategies was determined to be overwhelming. Patients and families frequently acknowledged poor compliance with the strategies. Further probing indicated it was difficult for patients to choose which strategy to use and in which situations. This issue is typical of executive dysfunction. We developed a more practical approach to minimize options and provide two or three focused strategies to patients and their caregivers. Additionally, we saw a need for caregiver education regarding executive dysfunction and its effect on behavior.

Purpose: To determine if a change in the manner of delivery, content of intervention, and specific caregiver training improved compliance with speech pathology management of dysarthria and dysphagia.

Method: Patients and families at a multidisciplinary ALS clinic were provided with two to three focused, individualized goals to manage dysarthria and dysphagia. Patients were informally surveyed at their next clinic to determine compliance with the strategies provided. Additionally, caregivers were educated with verbal and written material regarding executive function disorders and management strategies (e.g. introducing topic shifts in conversation).

Results: Informal survey revealed patient compliance with strategies to be 40%. Anecdotally, caregivers reported that patients used strategies more frequently when they were given cues. Many caregivers also reported they recognized executive function disorders in activities of daily living and were able to apply the management strategies to these

settings. This reportedly improved communication and reduced frustrations between patient and caregiver.

Discussion: Although compliance issues remain, patients did utilize speech and swallowing strategies more frequently when specific strategies were targeted for individual patients. More importantly, caregivers had a better understanding of executive function deficits, were able to manage behaviors and to communicate more effectively with the patients. The prevalence of executive dysfunction in this population suggests the need for modification of intervention methods and strategies across the multidisciplinary field to enhance patient compliance.

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P46 TREATMENT OF COGNITIVE IMPAIRMENT IN ALS

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Background: Cognitive impairment in ALS is known and is correlated with pathological and morphological findings. Therapeutic approaches have not yet been systematically studied.

Objective: This meta-analysis evaluates the diagnostic tools and the results concerning the extent and the character of cognitive impairment. The data of a pilot study with rivastigmine in ALS are presented.

Materials and methods: Studies of cognition in ALS published between 1996 and 2006 were acquired and sorted with regard to the design, the prospectivity, control and complexity of the studies, the selection of the control sample, the length of the observation period and the statistical revision.

The newly acquired data from this meta-analysis were reviewed in terms of possible therapeutic approaches and the therapeutic concepts for cognitive impairment were deduced. Ten consecutive patients with ALS and cognitive impairment were treated with rivastigmine.

Results: The best fit diagnostic tools for the ALS tests seem to be those that cover verbal and executive functions. A scale of depression is essential. This meta-analysis, which analyses studies with cohorts of about 30 patients with the aid of these tools, shows a highly significant prevalence of cognitive impairment in 30–50% of the patients with ALS. There is a distinct trend towards characterization of cognitive impairment in terms of a frontotemporal dementia. The selection of patients in terms of bulbar and extremity involvement shows unhomogeneous results. There is overall evidence for a stronger development of cognitive impairment in bulbar patients. Cognitive impairment is seen in about 40% of our own patients. The first results of the pilot study suggest a controlled study should be performed.

Discussion and conclusions: The pattern of cognitive impairment suggests a therapeutic approach analogous to that used in frontotemporal dementia. Central cholinesterase inhibitors or alternatively memantine are worth discussing. Our preliminary data suggest a positive effect of rivastigmine in ALS. The considerable extent of cognitive impairment especially in the bulbar form is to be considered clinically relevant. It has yet to be discussed how far the dementia affects the prognosis of ALS through lack of compliance in vital therapy decisions, or vice versa if successful therapy of cognitive impairment improves prognosis and quality of life.

We advise, on the basis of the present data, diagnosis and individual therapy of cognitive impairment. The extent of the coexistent or consecutive depression also has to be diagnostically and therapeutically considered.

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P47 FRONTOTEMPORAL DEMENTIA WITH ALS IN AKITA

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Background and objectives: Cognitive impairment in ALS has been reported to vary from 5 to 75% of patients. Recently, reports of ALS with dementia (ALS-D) have been increasing in the context of frontotemporal dementia (FTD) and Yuasa-Mitsuyama type-ALS. We have experienced ALS-D patients with severe dementia in Akita. Here we describe the clinical features and neuroimaging of the patients.

Methods: Of 75 ALS patients in our database, we chose 10 patients with marked dementia and compared them with 10 ALS patients without dementia (ALS-C) in terms of age of onset, initial symptom, neurological signs especially muscle stretch reflex, the type of dementia, HDS-R score, time course, and neuroimaging.

Results: Sixteen (22%) of 75 ALS patients showed severe dementia. The mean age of onset was 63 and 68 years in 10 patients of ALS-D and 10 with ALS-C, respectively. The gender ratio was 1:1 in both; the initial symptoms varied (dementia in three patients and weakness in seven patients in ALS-D); the scores of the HDS-R test were 10/30 (ALS-D) and 26/30 points (ALS-C). Character change, disinhibition, loss of insight, and aphasia (paragraphia, decreasing of speech) were recognized during the course of ALS-D. The average length of time between onset of weakness and dementia was 16 months. All patients had hyperreflexia and seven patients showed Babinski sign. The duration from onset to death or continuous ventilation in ALS-D was shorter than in ALS-C: 23.6 ± 14.6 and 36.2 ± 20.3 months, respectively. On CT or MRI, severe frontotemporal atrophy with rapid progression was remarkable in six patients. SPECT

showed the reduction of blood flow even in the early stage of ALS-D patients without atrophy sign.

Discussion and conclusions: ALS-D in Akita presented severe dementia and a rapid course of ALS with pyramidal signs, which are atypical both in Yuasa-Mitsuyama type ALS and FTD with motor neuron disease. Pathological and immunocytochemical investigation is awaited.

P48 COPING STRATEGIES IN A POPULATION OF 50 ALS PATIENTS

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Background: Coping has a strong influence on the quality of life of amyotrophic lateral sclerosis (ALS) patients and carers (1), and is one among the most relevant components in the assessment of subject psychological status.

Objectives: In this study, we evaluated twice over time specific ALS patients' coping strategies, in order to observe how coping strategies evolve with patient ageing and increasing physical impairment.

Methods: We used a translation of the MND Coping Scale (2), a questionnaire of 22 items concerning behaviours and attitudes strictly related to six factors representing particular coping strategies: 1) Support; 2) Positive Action; 3) Independence; 4) Avoidance/Venting; 5) Information Seeking; 6) Positive Thought. The scale was submitted twice to 50 ALS patients (19 female, 31 male; mean age at first testing 61.5 years). Second testing took place one year after the first test. Patients were divided into three age groups (Age 1, 29–55 years; Age 2, 56–74 years; Age 3, 75–86 years) and in three classes of illness severity (according to ALS-Functional Rating Scale (ALS-FRS): Class 1, 30–40; Class 2, 20–29; Class 3, 0–19).

Results: At first testing, we found significant ($p<0.05$) differences in Factor 2 between Age 1 and Age 3 groups and between Class 1 and Class 3: older and more impaired patients were less active than others. As far as second testing was concerned, many significant differences emerged between the ALS-FRS classes: on Factor 2 between Class 2 and Class 3 ($p<0.05$) and between Class 1 and Class 3 ($p<0.001$); on Factor 3 between Class 3 and both Class 1 and 2 ($p<0.001$); on Factor 4 between Class 1 and Class 3 ($p<0.05$); and on Factor 6 between Class 1 and Class 3 ($p<0.05$). As might be supposed, increased physical impairment seems to disadvantage adaptive coping strategies, but on the other hand we did not find significant differences by comparing first testing data with those from second testing. Finally, at second testing, male patients appeared to be more independent than females ($p<0.05$).

Discussion: Understanding the evolution of ALS patient coping strategies can be the first step to optimizing psychological intervention. Without forgetting the unique features of each patient, the identification of common trends in the development of patients' behaviour and attitudes could help us to reinforce those strategies that often tend to weaken along with increasing clinical disability and ageing of patients.

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P49 EVALUATION OF PSYCHOLOGICAL STATE IN ALS PATIENTS USING THE PROFILE OF MOOD STATES

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Background: Psychological problems frequently occur in patients with amyotrophic lateral sclerosis (ALS), including depression, anxiety and fatigue. Understanding the psychological status in ALS is important for the comprehensive management of patients. The Profile of Mood States (POMS) is an instrument composed of six mood scales that were developed using clinical populations to analyse mood and anxiety (1). Before now, no psychological evaluation has been reported using the POMS scale in ALS patients.

Objectives: To evaluate the status of mood and anxiety in patients with ALS.

Methods: Thirty patients with ALS in our hospital (15 men and 16 women, average age at examination 59 ± 25 years old, men 58 ± 29 years old, women 60 ± 21 years old, average disease duration 32 ± 72 months) were evaluated. We (neurologists, medical coordinator and clinical psychologist) performed psychological evaluation. Patients were interviewed with a questionnaire to evaluate their mood and anxiety using the Profile of Mood States (POMS), which assesses six subjective subscales rated on a five-point scale: tension-anxiety (0–36), depression-dejection (0–60), anger-hostility (0–48), fatigue-inertia (0–28), vigor-activity (0–32), and confusion-bewilderment (0–28). Their disease progression was estimated using the ALS Functioning Rating Scale (ALS-FRS). We performed correlation analysis between POMS score and age, gender, ALS-FRS and disease duration.

Results: The mean POMS score for all 30 patients was as follows: tension-anxiety (55.9 ± 21.2), depression-dejection (62.5 ± 24.9), anger-hostility (51.5 ± 18.9), fatigue-inertia (53.3 ± 20.5), vigor-activity (40.8 ± 20.1), and confusion-bewilderment (55.2 ± 25.5). Significantly greater frequency of tension-anxiety ($r=0.40$, $p=0.03$) and depression-dejection ($r=0.46$, $p=0.01$) was seen in

younger aged patients, and also significantly greater frequency of anger-hostility was seen in lower ALS-FRS score patients and more disabled ALS patients ($r=0.38$, $p=0.04$). No significant correlation between POMS scores and gender or disease duration was found.

Discussion and conclusions: Younger ALS patients have more tension, anxiety, depression and dejection. On the other hand, disabled ALS patients have more anger and hostility. We should provide mental care especially for both younger and severely disabled ALS patients according to their characteristic mental state.

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P50 CONCERNS OF JAPANESE CHILDREN OF PEOPLE WITH ALS

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Background: Children of people with disabilities are known to have their own concerns because of their unique experiences. The Motor Neurone Disease Association in the UK publishes booklets for children of parents who have motor neuron disease, and Tsuchiya reported Japanese children's burden as caregivers for their parents who live at home with ventilators. The burden of Japanese children of ALS patients is estimated to be higher than that of US and European countries because 10% of ALS patients stay at home with invasive ventilators and require 24 hour a day care, although the total amount of social service is limited.

Purpose: The purpose of this study is to discover the concerns of children of ALS patients at different developmental stages in order to develop a support system for them.

Methods: Semi-structured interviews were conducted with seven ALS patients with ventilators at home and nine family members. The conversations with the patients were facilitated by the family members using transparent boards and lip reading. The family members included two wives, one elder sister, one nephew and four daughters. The interview was fully recorded with an IC recorder and transcribed to categorize the contents.

Results: 1) The ages of children of ALS patients were from 2 years to the 40s; 2) Concerns of children differed with age – understanding of the disease for school-aged children, emotional support for adolescents and physical burden for adults; 3) There was no support or service for children of ALS patients except support from family members and home helpers.

Discussion: Children of ALS patients showed common concerns with the children of parents with physical disabilities and siblings of individuals with disabilities. The children of ALS patients require support in the form of information, emotional support and care burden support in a manner appropriate to disease development.

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THEME 4 DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P51 CLINICAL FEATURES OF ALS IN JAPAN FROM THE REGISTRATION SYSTEM OF INTRACTABLE DISEASES

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Background: Nationwide registration of the 45 intractable diseases is conducted by the Ministry of Health, Labour and Welfare in Japan. ALS is one of these diseases. The registration system was started in 1972, and its principal mission has been public expenditure for the medical expenses of patients with the intractable diseases. In 2003, the format of the medical certificate for registration of each disease was revised substantially, and, in 2004, the guideline for research use of the data from the registration system was established.

Objectives: To describe the clinical features of the ALS patients in Japan using the data from the nationwide registration system of intractable diseases.

Methods: We analysed the data from the initial and renewal registration of 7463 ALS patients from 2003 to 2005. All of the patients provided written informed consent for the research use of the registration data, and the anonymity of the data was strictly secured. We implemented the ethics guideline for epidemiology studies endorsed by the Japanese government.

Results: The proportion of male and female patients was 60.4% and 39.6%, respectively. The mean age at onset in the patients of initial registration was 65.1 $\pm$ 11.0 years. The initial symptoms were weakness of upper extremities in 47.1%, dysarthria in 39.5%, weakness of lower extremities in 35.2%, dysphagia in 22.9% and dyspnea in 5.9%. Overlap among the groups existed. Of all the patients, 8.5% were at work or in school attendance, 62.7% were under home care and 28.8% were hospitalized. A gastrostomy tube was used by 33.1% of the patients, 8.6% used nasogastric feeding, 5.7% used non-invasive positive pressure ventilation (NIPPV) and 34.3% continued tracheostomy positive pressure ventilation (TPPV). The mean duration from introduction of TPPV was 3.78$\pm$3.61 years. Ophthalmoplegia, vesicorectal disturbance and dyshidrosis were seen in 2.5%, 7.9% and 3.3% of the patients without TPPV, respectively. However, ophthalmoplegia, vesicorectal disturbance, and dyshidrosis were observed in 21.4%, 21.2%, and 8.4% of the patients, respectively, with duration from introduction of TPPV of greater than three years.

Discussion and conclusions: A large portion of the ALS patients in Japan are in advanced stages. The ratio of the patients on TPPV is much higher than those of the USA and EU. The data from the Japanese registration system of intractable diseases may provide beneficial information on the nature of ALS patients in Japan and may describe the clinical state of patients on TPPV in a large number of cases.

P52 PROGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS IN SOUTHERN ITALY: RESULTS FROM A POPULATION-BASED REGISTRY, SLAP (SCLEROSI LATERALE AMIOTROFICA-PUGLIA).

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Objective: To evaluate survivorship and the prognostic value of clinical factors in a population-based cohort of amyotrophic lateral sclerosis (ALS) incident cases.

Background: Most of the data on ALS natural history have been obtained by retrospective studies based on clinical series enrolled by ALS referral centres. Recently, population-based studies have provided prospective prognostic data but it is still not clear if El Escorial (EEC) diagnostic categories have prognostic value and whether advances in ALS therapy such as riluzole and non-invasive ventilation have improved survivorship.

Methods: The source of cases for the study was a prospective population-based registry established in Puglia, southern Italy. The diagnosis and the classification of cases were based on EEC criteria. Cases diagnosed as having ALS during 1998–99 were enrolled and followed up until death or 30 June 2004.

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Results: We identified 130 ALS incident cases; four patients were lost to follow-up. Median survival time from first symptom and from diagnosis was 28 and 18 months, respectively; survival rates at five years from first symptom onset and at four years from diagnosis were approximately 30%. Clinical course of suspected ALS was similar to that of definite ALS. Cases with predominant upper motor neuron (UMN) involvement, particularly among spinal onset ALS, presented a longer median survival time from onset and from diagnosis (45.2 and 23.8 months, respectively), associated with lower mortality rates (four years from diagnosis, 57%). Multivariate analysis revealed that advanced age and bulbar or generalized onset were independent predictors of adverse survival. Multivariate models after stratifying patients according to site of first symptoms (bulbar vs spinal) showed that age and site of onset were independently related to outcome, as no effect of age on survival was observed among bulbar ALS cases (HR:1.0.2; 95% CI 0.97–1.06; $p=0.4$).

Conclusions: In this incident case series, survival times are consistent with other population-based studies on natural history of ALS. Advanced age and bulbar onset of symptoms are significant indicators of poor prognosis while EEC category at time of diagnosis does not predict survival.

P53 PROGNOSTIC FACTORS IN AMYOTROPHIC LATERAL SCLEROSIS WITHIN SOUTHWESTERN ONTARIO, CANADA

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Background: Accurate prognostic information is important to provide to patients diagnosed with amyotrophic lateral sclerosis. Limb onset, young age and male sex have been cited as good prognostic factors for patients diagnosed with ALS. Most of the studies in the literature which have addressed prognosis are from the United States and Italy. There is little recent data available for the Canadian population.

Objectives: To determine important prognostic indicators in the population of southwestern Ontario, Canada.

Methods: Our ALS clinic database, which documents prospectively collected information on our ALS clinic patients, was evaluated for patients seen from 1991 to April 2006. Univariate survival modelling was performed using Kaplan Meier survival analysis. Multivariable Cox proportional hazard modelling was used to evaluate potential prognostic factors including sex, age, occupation, smoking and site of symptom onset. Hazard ratios were determined for each variable.

Results: The database consisted of 791 individuals with definite or probable ALS. The mean age of onset was 59.6 ± 12.9 years, with a range of 17 to 89 years. Mean age of onset was similar for onset prior to 1996 and onset after 1996 (59.1 and 60.2 years, respectively). The median and mean survival time from symptom onset to death or permanent assisted ventilation was 33.5 months and 50 months, respectively. Thirteen individuals (4.8%) survived for more than 10 years. Univariate analysis demonstrated that the survival was worse for age >45 years (median 31.3 vs. 62.3 months) and bulbar onset (median 28.0 vs. 37.0 months). Female sex was not found to be a negative prognostic factor in univariate analysis. The best prognostic category was very young males with onset prior to age 30 (median survival, 89.9 months). Cox proportional hazard modelling found that older age, bulbar onset, smoking, and onset after 1996 were negative predictive factors when other factors are controlled for. Sex and occupation were not significant prognostic factors.

Discussion: This study will allow better prediction of prognosis in our patients. The findings of this study are generally comparable to previously published survival evaluations with the exception of the finding that sex was not found to be a significant prognostic indicator. The finding of worse prognosis with more recent onset, despite controlling for other variables, has also been suggested in an evaluation of a Scottish database. A plausible explanation for the appearance of decline in survivorship over time is that it is likely that a statistical artefact is created by a greater degree of censoring in the more recent diagnostic group.

P54 MOTOR NEURON DISEASE IN NORTHERN TAIWAN

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Introduction: Motor neuron disease is caused by degeneration of neurons in the motor cortex, cranial nerve nuclei, and anterior horn cells. Upper and lower motor neurons may be affected but there is no sensory loss or sphincter disturbance. The cause is unknown.

In Taiwan, motor neuron disease belongs to group of rare diseases. Although there is a Taiwan MND Association to help patients with motor neuron disease, the actual prevalence in Taiwan is unknown. We collected data on patients admitted to a medical center in northern Taiwan who were diagnosed with motor neuron disease and analysed the epidemiology of these patients.

Methods: We retrospectively recorded patients admitted to a medical center in northern Taiwan with a final diagnosis of motor neuron disease from January 1988 to November 2005. We analysed age, sex, and duration from onset of symptom to diagnosis, type of motor neuron disease, treatment, number of hospitals patients visited and prognosis.

Results: During the 18 years of the study, 230 patients were admitted to our hospital with a discharge diagnosis of motor neuron disease. There were 85 female patients and 145 males, a sex ratio of 1.7. The average age during

admission was 56.6 years and there was no significant difference in age between females (52.8 years old) and males (58.8 years old). The youngest patient was one year old and the eldest was 88 years old. Most of patients were diagnosed with amyotrophic lateral sclerosis. Only patients diagnosed with amyotrophic lateral sclerosis can be prescribed riluzole under application from clinical doctors. Most patients were admitted to a neurology ward. There were still a few patients admitted to an internal medicine ward, pediatric ward or surgery ward under other causes and diagnosed under neurology consultation. Average admission time was around 7.8 to 44.1 days and the longest duration of admission was 227 days. In the past many patients had visited other hospitals for evaluation. There was a slight elevation in the number of patients admitted (the fewest, one patient in 1989 and the most, 28 patients in 2004).

Conclusions: Patients diagnosed with motor neuron disease in northern Taiwan had a similar sex ratio as reference. Although there were very young and old patients, the average age at diagnosis was 56.6 years. It is worth noting that many patients had visited other hospitals for a second opinion. There is an increasing frequency for a non-neurologist to transfer patients to a neurologist under suspicion of motor neuron disease. We should continue to follow up patients diagnosed with motor neuron disease and consider how to improve their quality of life under an incurable disease.

P55 THE AGE AT ONSET OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH BULBAR DYSFUNCTION OR FACIAL DIPLEGIA IS HIGHER THAN THAT OF ALS PATIENTS WITHOUT THESE ABNORMALITIES

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Background: In the study of autopsy cases of amyotrophic lateral sclerosis (ALS), the age at onset of the disease was significantly higher for the bulbar onset form than for the limb onset form.

Objctives: The aim of this study was to investigate the correlation between the neurological signs and the age at onset in patients with ALS.

Methods: We studied 29 consecutive patients (19 men and 10 women, mean age 62.8 years old; range 38–85 years) who were admitted to the Department of Neurology and Neuromuscular Disorders, Oita University Hospital, between January 1999 and December 2004. We evaluated the clinical manifestations and the mean age at onset of these patients with each neurological sign or symptom. According to the revised El Escorial criteria (Airlie House Conference, 1998), all patients were clinically definite for ALS. Differences between the variances were tested using the Mann-Whitney *U*-test as a non-parametric method. The level of statistical significance was set at $p<0.05$.

Results: Despite no significant difference in the duration of illness, the mean ($\pm$SE) age at onset in ALS patients with bulbar dysfunction (dysphagea and/or dysarthria) was significantly higher than that of ALS patients without this sign (14 cases vs. 15 cases, 67.4 ± 2.3 vs. 58.5 ± 2.5; $p<0.05$). The mean ($\pm$SE) age at onset in ALS patients with facial diplegia was significantly higher than that of ALS patients without this sign (16 cases vs. 13 cases, 68.3 ± 1.7 vs. 56.1 ± 2.8; $p<0.01$).

Discussion and conclusion: The age at onset of ALS patients with bulbar dysfunction or facial diplegia is higher than that of ALS patients without these abnormalities.

P56 BLINK REFLEX HABITUATION IS REDUCED IN THE EARLY STAGE OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: The assessment of Blink Reflex Habituation (BRH) is a sensitive method to evaluate the influence of suprasegmental structures on brainstem interneurons. BRH could be used as a marker of non-compressive upper motor neuron (UMN) dysfunction. Previous studies of BRH in patients with amyotrophic lateral sclerosis (ALS) are scarce and contradictory.

Objective: The aim of the present study was to assess the BRH by using paired electrical stimuli in patients with early stages of ALS.

Methods: We studied 21 patients with definitive diagnosis of ALS (12, bulbar onset; 9, limb onset), with less than one year from symptom onset, and 18 age-matched healthy controls. A typical evoked potential study of the BR was employed. The effects of paired stimulation on R1 and R2 components, with interstimulus intervals (ISI) of 100, 250, 500 and 1000 milliseconds (ms) were compared in both groups. BRH was defined as the percentage of change of the amplitude of the test response compared to the conditioning response ((+) increased; (−) decreased).

Results: R1and R2 latencies and R1 amplitude elicited by single stimulation were similar in patients and controls; however, ALS patients showed significantly reduced R2 amplitudes compared to healthy subjects (280 (140) uv vs. 400 (81) uv). The most significant differences in BRH between groups were detected with an ISI of 250 ms. The amplitude of test R1 was facilitated to a significantly greater extent in the patients (+35.5% (15.4)) than in the controls (+10.2% (5.2)), $p<0.05$. Conversely, the change in amplitude of test R2 was significantly less in ALS patients (−15.5% (14.8)) compared to healthy volunteers (−71% (24.2)), $p<0.01$.

Conclusions: These findings demonstrate that patients with ALS, even at early stages of the disease, have a reduced habituation of the electrically induced blink reflex.

These changes may relate to increased suprasegmental facilitatory influences on brainstem interneurons. The evaluation of BRH could provide evidence of subclinical UMN involvement, which might help to establish an earlier diagnosis of ALS, particularly in those with predominantly LMN dysfunction.

P57 ELECTROPHYSIOLOGICAL ABNORMALITIES IN ALS ACCORDING TO THE EL ESCORIAL CRITERIA

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Background: A wide variety of clinical features may be present early in the disease course of ALS and this makes diagnosis difficult. Since there is no reference test for diagnosing ALS, in 1990 a set of diagnostic criteria, the El Escorial criteria, was put forward by the World Federation of Neurology. In 1998 the El Escorial criteria were revised. This diagnostic set consists of a combination of clinical and electrophysiological abnormalities. The added value of electrophysiological studies in the El Escorial criteria has, however, not yet been validated.

Objective: To describe the contribution of electrophysiological signs in diagnosing ALS in clinical practice.

Methods: All patients who visited our outpatient clinic were included in our study and classified according to the revised El Escorial criteria. Electrophysiological studies were performed in patients who fulfilled the criteria for possible, probable or definite ALS, based on clinical signs of motor neuron dysfunction.

Results: In 2003, 147 patients visited our outpatient clinic. Of these, 84 patients were diagnosed as having ALS. According to the El Escorial criteria, one (1%) patient had definite ALS, 24 (29%) patients had probable ALS and 59 (70%) patients had possible ALS. After performance of electrophysiological studies, nine (15%) of the patients who were clinically diagnosed as having possible ALS moved up a diagnostic category to probable laboratory-supported ALS. Discrepancies were found between clinical signs of LMN dysfunction and electrophysiological features in bulbar and thoracic regions.

Conclusion: Our study shows that electrophysiological studies may have a limited contribution to the El Escorial criteria. The true added value of electrophysiological studies should be investigated in well designed diagnostic research.

P58 APPLICATION OF THE NEUROPHYSIOLOGICAL INDEX IN ALS PATIENTS

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Background: Neurophysiological Index (NI) is a neurophysiological measure made up from the M wave amplitude, the distal motor latency and the F-wave frequency in ulnar nerves. Several studies have suggested that NI could be used as a sensitive measure of changes during the course of ALS. However, NI has several limitations, in that it was only derived from the ulnar nerve in previous studies and is less valuable when the patient is in the terminal or very early stages of the disease. This study evaluated the validity of NI, obtained from the median and posterior tibial nerves.

Methods: Twenty-nine consecutively recruited patients with ALS were studied prospectively. Patients with other medical conditions (e.g. diabetes mellitus, polyneuropathy, and nerve entrapment) were excluded. NI obtained from ulnar, median and posterior tibial nerves were recorded and ALS-FRS, Norris scale, and Appel scores were also recorded serially in some patients. NI data were compared with ALS-FRS, Norris scale and Appel scores.

Results: The mean age of the patients was 48.9 years; 19 patients were male and 10 patients were female. Eighteen patients were probable ALS and 11 patients were definite ALS. Eight patients were bulbar onset type and the others were limb onset type. NI, obtained from each nerve, showed a significant correlation with other data from various scales in ALS patients.

Conclusion: In our study, we proposed that NI obtained from median and posterior tibial nerves should also be available to measure the changes during the course of ALS and its treatment.

P59 NEEDLE ELECTROMYOGRAPHY OF THE RECTUS ABDOMINIS MUSCLE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background and objective: Needle electromyography (EMG) of the rectus abdominis muscle (RA) was performed to assess thoracic involvement in the diagnosis of amyotrophic lateral sclerosis (ALS).

Methods: EMG of the RA was performed in 67 patients with sporadic ALS and 110 healthy controls. EMG parameters included the presence of spontaneous activity,

configuration of motor unit action potentials (MUAP), and recruitment pattern of motor unit potential.

Results: MUAP RA parameters in controls were: duration (9.95 ± 1.13) ms, amplitude (373.78 ± 56.46) μV, and polyphasic waves $11.75 \pm 3.26\%$. In comparison, ALS patients displayed: duration (13.02 ± 1.30) ms, amplitude (537.19 ± 159.04) μV, and polyphasic waves $31.19 \pm 8.84\%$. Significant differences in MUAP parameters, spontaneous potentials and reduced interference patterns were noted between ALS patients and healthy controls. Additionally, we found that active denervation was more frequent in RA in ALS patients with dyspnoea than those without dyspnoea.

Discussion: Our data suggested that conventional needle EMG of RA was a valuable electrophysiological method to assess the clinical and subclinical involvement of the lower motor neuron, in the thoracic region, in ALS patients.

P60 PROSPECTIVE STUDY OF MOTOR FUNCTION AND MOTOR UNIT NUMBER ESTIMATION IN SPINAL MUSCULAR ATROPHY

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Background: Motor unit number estimation (MUNE) has been used to measure the number of functional motor units in spinal muscular atrophy (SMA). In previous studies, MUNE did not correlate with strength in proximal muscles (1), but correlated with clinical phenotype (SMA types I, II, or III) and with a five-step functional scale (2).

Objective: To study the relationship between MUNE and clinical phenotype using two more detailed motor function measures that have been independently validated in the SMA population (3,4).

Methods: Forty-one subjects with SMA types I ($n=5$), II ($n=20$) and III ($n=16$), aged seven months to 45 years, were prospectively evaluated as part of a comprehensive, longitudinal study. Using the multiple point stimulation technique, MUNE was measured in the hypothenar muscle group at baseline (in all subjects) and at six months (in 19 subjects to date). In subjects older than two years, motor function was assessed using the Gross Motor Function Measure (GMFM) (4) and/or the Hammersmith Functional Motor Scale (HFMS) (3).

Results: MUNE values differed between the three clinical phenotypes, but showed some overlap. The lowest values were seen in SMA type I (mean=6.1, interquartile range, IQR=0.8), intermediate levels in type II (mean=20.0, IQR=10.1), and highest levels in type III (mean=96.5, IQR=97.8). There was a significant correlation between MUNE and gross motor function score for both the GMFM ($r=0.71$), and the HFMS ($r=0.79$). In the 19 subjects with follow-up MUNE, there was no significant change over six months (mean change 1.5, median change 0.7, SD 7.0).

Discussion and conclusions: MUNE correlates with two independent gross motor function scales. This suggests that MUNE is a potentially valid biomarker reflecting disease severity. Further research is needed to study its utility as secondary outcome measure for SMA clinical trials.

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P61 PREDICTIVE USE OF I-123-MIBG SPECT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Autonomic dysfunction is generally not considered to be a typical clinical feature of amyotrophic lateral sclerosis (ALS). However, previous studies revealed subclinical abnormalities of the autonomic system in patients with ALS. Sympathetic innervation of the heart was shown by I-123-metaiodobenzylguanidine-single photon emission computed tomography (MIBG-SPECT), which has been used for evaluation of cardiac autonomic function of neurodegenerative diseases such as PD, DLB.

Objectives: To investigate the autonomic nervous function of patients with ALS using MIBG-SPECT and to elucidate the relation between MIBG-SPECT and other autonomic examination.

Methods: Twenty-six consecutive patients diagnosed with definite or probable ALS were examined by MIBG-SPECT and ECG ($CV_{R\text{-}R}$), and serum catecholamine levels were determined.

Results: 1) Washout rate (WR) of cardiac MIBG-SPECT was significantly increased in all ALS patients compared with controls ($p<0.05$). The heart/mediastinum ratio of the early phase and the delayed phase in all ALS patients is not

significantly different in comparison with controls. 2) WR of cardiac MIBG-SPECT was significantly increased in patients with ALS where the period from onset to first medication was within one year compared with those where the period was over one year ($p<0.05$). 3) WR of cardiac MIBG-SPECT was significantly increased in patients with ALS whose progression rate (the delta FS) was over 1, compared with patients with ALS where the delta FS was under 1 ($p<0.05$). The relation between MIBG-SPECT and other autonomic examination is not significant.

Discussion and Conclusions: Increased WR of cardiac MIBG-SPECT suggested sympathetic nervous hyperfunction. This finding supports the previous study that autonomic dysfunction of patients with ALS is due to sympathetic nervous hyperfunction. WR of cardiac MIBG-SPECT was significantly increased in the rapidly progressing ALS patients at the time of diagnosis. It is reported that progression rate at time of diagnosis in each patient is associated with prognosis in Japan. WR of cardiac MIBG-SPECT could be used in the follow-up of individuals with ALS in therapeutic trials. In future, it is necessary that the prognosis of these patients with ALS is evaluated.

P62 [^{11}C]-FLUMAZENIL PET IN PLS: COMPARISON WITH SPORADIC AND HOMOZYGOUS 'D90A' SOD1 ALS

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Background: Primary lateral sclerosis (PLS), despite its consistently slower progression and *sine qua non* of clinically pure upper motor neuron involvement, is generally accepted as a rare extreme of the clinicopathological spectrum of ALS/MND. Patients homozygous for the 'D90A' SOD1 gene mutation (homD90A) also have a consistent phenotype that is associated with slower disease progression (mean 14 years). [^{11}C]-flumazenil PET has been used to demonstrate a different pattern of cerebral pyramidal neuronal loss/dysfunction in sporadic (SALS) and homD90A ALS patients when both were compared with healthy controls (1).

Objectives: To compare the pattern of reduction in the cerebral binding of [^{11}C]-flumazenil in PLS patients with that seen in SALS and homD90A patients; first, when all patient groups are compared separately with healthy controls, and secondly when comparing the PLS patients directly with the SALS and homD90A groups.

Methods: Four PLS, 24 SALS and 10 homD90A patients underwent [^{11}C]-flumazenil PET of the brain, and results for each group were compared separately with a group of 24 healthy controls. The PLS patients were then compared directly with the SALS and homD90A groups in turn.

Results: The pattern of reduction in the cerebral binding of [^{11}C]-flumazenil in PLS patients compared with controls was similar to that seen in SALS patients, involving predominantly the motor cortex bilaterally and extending to the right parietal cortex. Additional reductions were seen in the margin of the left superior temporal lobe and anterior cingulate gyrus in the PLS group. When the SALS and homD90A groups were directly compared with the PLS patients, relative decreases were found bilaterally in the anterior frontal and orbitofrontal regions of the non-PLS groups, and these were most marked in the homD90A patients.

Conclusions: These [^{11}C]-flumazenil PET brain findings support the concept that PLS is part of the clinicopathological spectrum of sporadic ALS. They also provide evidence that PLS patients may have a relative preservation of frontal lobe neuronal pathways (or delayed progression of disease in these areas) compared to other ALS patient groups. Detailed comparative cognitive studies, focusing on frontal lobe tasks, may support these observations.

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P63 WHITE MATTER BRAIN INJURY AS MEASURED BY MAGNETIZATION TRANSFER MAY CORRELATE WITH DISEASE DURATION

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Background: Primary lateral sclerosis is an idiopathic, non-familial neurodegenerative disorder of the upper motor neurons whose pathophysiology is still unknown. Although a few studies have revealed brain atrophy most notable in the cortical and subcortical portions of the precentral area, there is very little information on the integrity of the remaining brain. Magnetization transfer (MT) imaging is a novel MRI technique that facilitates the quantitative evaluation of the integrity of the brain parenchyma. The MT ratio (MTR) images have advantages over conventional T2- and T1-weighted images in that they have greater pathological specificity and are intrinsically semi-quantitative. A low MTR reflects a reduced exchange of magnetization between macromolecules and surrounding water molecules. MTR has revealed abnormalities in a number of conditions, including Alzheimer's disease, multiple sclerosis, Parkinson's disease, and stroke (1). In addition, a reduced MTR has been reported in the corticospinal tract of patients with amyotrophic lateral sclerosis (2).

Objectives: The aim of this study was to assess whether MTR in the centrum semiovale or precentral gyrus was related to disease severity and/or disease duration.

Methods: Patients with a clinically definite diagnosis of PLS were recruited from the neuromuscular clinic. They underwent a routine MR examination on a 1.5 T MR scanner using a phased-array head coil. The MRI protocol included an MT on-and-off sequence so as to be able to calculate MTR. The MT images were acquired with a pair of 3D spoiled gradient echo sequences with a TR of 33, a TE of 9 ms, an excitation angle of 15°, a FOV of 250 mm, and a 256×256 matrix. Three mm-thick slices through the whole brain were acquired with (Sat) and without (NoSat) saturation. A percent difference image, hereafter called the MTR image, was calculated on a voxel-by-voxel basis according to the equation: MTR=(1 − Sat/NoSat) $\times 100$, for those voxels that exceeded a background noise threshold. Mean MTR values were obtained from regions of interest (ROI) manually drawn. Spearman's rho correlations were calculated.

Results: Six patients have been scanned to date. There was a trend for MTR median in the centrum semiovale to decrease with disease duration, $r=-0.543$, $p=0.13$. The trend was less evident in the precentral cortex, $r=-0.429$, $p=0.2$. There was no correlation with age or severity of disease.

Conclusions: Our preliminary results suggest that MTR could be useful for assessing brain integrity and that there is ongoing damage that is more evident in the white matter.

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P64 REDUCED ANISOTROPY OF THE CORTICOSPINAL TRACT DETECTED BY CONVENTIONAL DIFFUSION WEIGHTED MR-IMAGES (DWI) IN ALS

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Background: Diffusion tensor imaging (DTI) is able to reveal reduced anisotropy along the corticospinal tract (CST) in ALS patients. However, DTI requires specialized equipment not available in most clinics. Therefore, only a small number of patients in a few centres have been studied until now.

Objectives: We investigated whether common diffusion weighted images (DWI) are also suitable to detect abnormalities of CST anisotropy in ALS patients.

Methods: We examined 35 ALS patients (18 males, 17 females; mean age 61.6 ± 9.9 years, median duration of disease 10 months) and 19 controls (10 males, 9 females; 57.7 ± 12.2 years). MRI scans were recorded with a 1.5T MR device (Sonata, Siemens) with standard commercial diffusion weighted sequences. We quantified CST signal intensity in five levels along the CST. Signal values in right to left, and anterior to posterior direction, were averaged. We calculated the ratio of this mean value to the signal value of the cranio-caudal direction and named it the anisotropy ratio (AR). Due to the direction of the CST in cranio-caudal direction, AR should be high in the case of intact CST (high anisotropy) and low in the case of degenerated CST (low anisotropy).

Results: At two levels of the internal capsule (upper and lower IC, posterior limb), AR was significantly reduced in ALS patients compared to controls (upper IC right: ALS 2.04, control 2.16, $p=0.088$; upper IC left: ALS 2.07, control 2.23, $p=0.03$; lower IC right: ALS 1.67, control 2.23, $p<0.005$; lower IC left: ALS 1.75, control 2.16, $p<0.005$). AR values at the level of the crus cerebri and pons were lower in ALS patients, but the difference was not significant. At the level of the precentral white matter, AR was significantly lower for the right medial ROI (ALS 1.41, controls 1.60; $p=0.032$), but not for the left medial and left and right lateral ROI.

Conclusion: At the level of the internal capsule reduced anisotropy is detectable with common commercial DWI in ALS. This pilot study has to be completed by a follow-up study. Possibly, calculation of the AR at the level of the interne capsule might be used as a simple marker of CST degeneration.

P65 IS CORTICAL EXCITABILITY ABNORMAL IN MND? A STUDY USING PHARMACOLOGICAL FMRI

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Background: Motor neuron disease (MND), also known as amyotrophic lateral sclerosis (ALS), is a progressive and ultimately fatal disease characterized by degeneration of corticospinal tract and spinal motor neurons. There is evidence that excitotoxity contributes to neuronal damage and death in MND. Excess glutamate induced cortico-motor neuronal excitotoxicity, and subsequent loss of recurrent collaterals of corticospinal tract pyramidal neurons that are known to activate intrinsic GABAergic interneurons or a primary loss of these GABAergic interneurons are possible mechanisms responsible for the altered intracortical balance between excitation and inhibition, observed during electropysiological and neuroimaging studies.

Objective: To investigate, at a synaptic level, the possible role of altered excitatory and inhibitory mechanisms in the pathophysiology of ALS using fMRI and intravenous challenge of a GABA-A receptor agonist midazolam.

Methods: Using functional imaging (fMRI) we studied seven patients with typical ALS and seven healthy controls matched for age and sex, while they performed a visually paced motor task. All the subjects were scanned while they received an intravenous challenge of normal saline and on a separate visit an infusion of midazolam.

Results: Task related functional changes were identified in motor cortical and subcortical regions. Following intravenous midazolam, direct group comparisons revealed a decreased BOLD response in the contralateral sensorimotor cortex, contralateral premotor area and supplementary motor areas in healthy controls. Furthermore, the BOLD response was increased in subcortical areas bilaterally and the cerebellum of healthy controls. In the ALS group, post midazolam, the BOLD response was increased in subcortical and cortical motor areas.

Conclusions: The suppression of BOLD signal seen in the cortical areas of healthy volunteers following midazolam challenge may be a result of direct GABA-A receptor mediated inhibition but may also be due to dampening down of glutamatergic output from the motor cortex. One of the consequences of this 'cortical suppression' might be an increased input from a parallel motor loop to the subcortical area reflected in a BOLD signal in this area. The lack of suppression of the BOLD signal in the ALS group following a midazolam challenge may be a consequence of loss of inhibitory GABAergic neurons in the motor cortex.

P66 DIFFUSION TENSOR IMAGING (DTI) AND MAGNETIC RESONANCE SPECTROSCOPY (MRS) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): A LONGITUDINAL STUDY

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Background: Looking for an objective marker for upper motor neuron involvement in amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND) patients, non-conventional magnetic resonance imaging (MRI) techniques, such as diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (H-MRS), have been recently employed.

Objectives: The aim of our work was to discover neuroimaging markers for progressive clinical impairment of ALS/MND patients.

Methods: We studied 32 ALS/MND patients, 13 with definite/probable (D/P) and 19 with possible (P) ALS, according to the El Escorial criteria. All subjects underwent brain MRI scans every four months by 1.5-T device (GE Signa Excite^{TM} HD, Milwaukee, USA). H-MRS was carried out by sampling rolandic regions by the single voxel technique. Diffusion fractional anisotropy (FA) and average apparent diffusion coefficient (avADC) were analysed along the cortico-spinal tract from grey and white prerolandic matter down to the bulbar pyramids. Patients' data were compared among each other according to progressive clinical impairment.

Results: Taking into account that our data are preliminary, we found a significant relationship between decreased FA values and increased clinical impairment over time in both all patients as a whole and the D/P ALS group only, for right prerolandic white matter, left brain peduncle and bilateral pons. Moreover, a significant correlation between enhanced avADC values and increased clinical impairment was observed only in D/P ALS patients for right prerolandic white matter and right posterior limb of the internal capsule. An inverse relationship between NAA/Cr ratio and clinical impairment was observed in H-MRS scans of both rolandic regions in all subjects (but only at right side in D/P ALS patients).

Discussion: Altered diffusion indices can be explained by a progressive cortico-spinal tract derangement due to motor fibre degeneration. Spectroscopic data, according to ALS pathophysiology, might be regarded as a marker for neuronal loss or dysfunction over time.

Conclusions: Our findings, although incomplete and preliminary, are encouraging and allow us to hope that H-MRS, but especially DTI, are useful tools in disease monitoring of patient groups, and therefore during clinical trials.

P67 DISCOVERY OF NEW CEREBROSPINAL FLUID PROTEIN BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Background: Confirming diagnosis of ALS or other motor neuron disease currently entails several months. Accomplishing this task rapidly would benefit patients so that they may begin appropriate therapy. Determing the nature and status of motor neuron disease may be realized by detecting biomarker proteins in patient cerebrospinal fluid (CSF). Using a systems biology approach we have analysed changes in gene and protein expression in the spinal cord tissue of a mouse model of amyotrophic lateral sclerosis (ALS) using gene ontology mapping (1). The results indicated that cellular communication was the largest category containing expression differences. One subcategory within this group includes proteins involved in cell adhesion. These are proteins associated with the extracellular matrix and establishment of cell-cell contacts. We hypothesized that changes in the expression of these proteins may be reflected in the CSF and serve as disease-related biomarkers.

Objectives: 1. Obtain profiles of CSF proteins from control and ALS patient groups. 2. Identify a panel of biomarkers that distinguish ALS from controls.

Methods: Patient and control CSF samples were analysed using proteomics methods. Albumin and immunoglobulins were removed using an Agilent immunodepletion column. The eluant was fractionated using single dimension sodium dodecyl sulphate-polyacrylamide gel electrophoresis (1D-SDS PAGE). Twelve gel slices were digested with trypsin and peptides extracted. Peptides were analysed by nanoscale liquid chromatography coupled to an ion trap mass spectrometer. Mass spectral data accumulated from multiple runs were then processed and searched against the human International Protein Index database to directly identify the proteins represented by the individual peptides.

Results: Relative protein abundance data from ALS compared to control samples was analysed using BRB-array tools. Class prediction algorithms were employed to discover biomarkers associated with ALS. Three of the seven identified proteins belong to the extracellular-matrix protein category supporting our hypothesis of detecting changes in these proteins in the CSF of ALS patients compared to controls.

Conclusion: Ongoing analysis of additional samples and validation of newly identified protein candidates using immunoassays will ultimately allow a panel of disease relevant biomarkers to be used in the diagnosis of motor neuron disease from patient CSF.

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P68 PROTEIN BIOMARKERS FOR DISEASE PROGRESSION DURING AMYOTROPHIC LATERAL SCLEROSIS

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Background: Biomarkers specific to motor neuron disease will aid in the diagnosis of disease and identify new therapeutic targets. Recent studies have identified individual or panels of protein biomarkers that represent putative ALS biomarkers. Beyond the use of biomarkers in disease diagnosis, protein biomarkers that can monitor disease progression would be quite useful as surrogate markers in clinical trials to test drug efficacy and to identify drug combinations that may provide the best treatment strategy.

Objectives: To perform mass spectrometry based proteomics on cerebrospinal fluid (CSF) collected over time from individual ALS subjects. By comparing the CSF protein profile during the course of disease we will attempt to uncover protein alterations that correlate to clinical signs of ALS disease progression.

Methods: CSF was collected every four to six months from a cohort of ALS subjects enrolled at the University of Pittsburgh School of Medicine ALS/MND clinic. Clinical measurements were performed at each clinic visit to evaluate disease progression. CSF was collected over a two-year time frame from each subject and analysed by mass spectrometry. Results were compared to the CSF profile of healthy control subjects.

Results: By comparing the CSF protein profile over time, we identified a group of protein peaks that exhibit alterations (increased or decreased levels) that correlate to disease progression within individual patients. While a few protein peaks were unique to specific patients, all contained a common set of proteins that changed during disease progression. However, each patient exhibited an individual rate of protein alterations over time that correlated with their individual rate of clinical disease progression. Finally, protein biomarkers identified for disease progression were largely distinct from diagnostic protein biomarkers.

Discussion and conclusions: Our results suggest that protein biomarkers for ALS disease progression can be identified in the CSF. Biomarkers for disease progression will aid in the development of effective drug treatments for ALS and other motor neuron diseases.

P69 ABSTRACT WITHDRAWN

P70 TOWARDS IDENTIFYING A MEANINGFUL BIOMARKER FOR ALS: A RETROSPECTIVE CLINICAL STUDY

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Background: There is no universally accepted biomarker that uniquely identifies patients with amyotrophic lateral sclerosis (ALS) though many have been proposed. Using SELDI proteomics, we previously identified down-regulation of a three-protein profile in patients with ALS (VGF fragment, cystatin C, and a 6.7kD protein yet to be identified) that is 95% accurate, 91% sensitive and 97% specific. One of these proteins is VGF (non-acronymic name given to a nerve growth factor inducible protein

instrumental in maintaining metabolic homeostasis). VGF is a member of the granin family of proteins (chromogranin A and B) that form neurosecretory granules. We have created an ELISA that quantifies VGF (entire molecule) and chromogranin A to begin validation that these proteins may indeed be true biomarkers for ALS. One requirement for a biologically meaningful marker of disease is that it varies with disease severity.

Objective: To determine if levels of VGF and chromogranin A (CgA) vary with disease severity in patients with ALS.

Methods: A retrospective survey was performed for all patients who participated in our ALS and motor neuropathy biomarker study between November 2004 and May 2006 (n=43). Selection criteria consisted of the following: El Escorial defined probable or definite ALS, cerebrospinal fluid (CSF) available for ELISA analysis, clinical examination with quantitative muscle strength measurements within four weeks of CSF acquisition. Patients were classified according to number of segments with clinical weakness from a total of three segments of the central nervous system (cranial, cervical and lumbar). Quantitative muscle testing was based on the MRC grading system out of a total of 200 possible points. Concentration of total VGF and CgA was calculated for each patient. Statistical analysis of muscle scores and concentration of VGF and CgA was performed using independent-measures t-tests.

Results: Out of 43 patients identified, 17 had ALS. The remaining 26 patients did not satisfy criteria for the following reasons: three had frontotemporal dementia, three had primary lateral sclerosis, and 20 had diseases other than ALS or did not have clinical measures available for review. Clinical weakness identified only in one segment occurred in 10 patients; weakness in two segments was identified in seven patients. Patients with two affected segments were significantly weaker (muscle testing score 149.8 vs. 183.3, p=0.018) and have significantly lower concentrations of VGF (4.47 vs. 5.48, p=0.038) and CgA (39.25 vs. 70.69, p=0.038) than those with only one segment affected.

Discussion and conclusion: In a retrospective study, we have demonstrated that potential biomarkers for ALS which we previously identified using SELDI proteomics are able to be quantitatively measured using ELISA. We further showed that there is a statistically significant reduction in concentrations of VGF and CgA in more severely affected patients suggesting that such markers may correlate with disease severity. These studies further support our findings that VGF and possibly CgA may be biologically significant biomarkers for ALS. Prospective studies are needed.

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THEME 5 THERAPEUTIC STRATEGIES

P71 DEXTROMETHORPHAN-QUINIDINE (DMQ) TREATMENT OF PSEUDOBULBAR AFFECT (PBA) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: ALS patients with pseudobulbar affect (PBA) were randomized to dextramethorphan-quinidine (DMQ) (n=70), DM (n=33) or Q (n=37) as published (1). DMQ effects were measured by the CNS-Lability Scale which contains self-rated questions to separately assess threshold and control of crying and laughing lability.

Objective: To determine whether DMQ, an effective treatment for PBA in ALS patients, works through adjusting the threshold, control or both for crying and laughing lability.

Methods: Outcomes for individual questions in DMQ, DM, Q and DM plus Q patient groups were analysed at d1, d15 and d29 after randomization and initiation of treatment. Comparisons were made using one-way analysis of variance with contrasts.

Results: DMQ significantly decreased, at d15 and d29, CT (−1.21, −1.07) (p<0.0001, <0.0001), CC (−1.22, −1.31) (p<0.0001, <0.0001) and CCP (−1.38, −1.20) (p<0.0001, <0.0001) compared with Q and CT (−0.93, −0.90) (p<0.0001, <0.0001), CC (−0.87, −0.93) (p<0.0001, <0.0001) as well as CCP (−0.97, −0.87) (p<0.0001, <0.0001) compared with DM and Q. DMQ significantly decreased, at d15, but not d29, LT (−0.61, −0.31) (p=0.0148, 0.1907), LC (−0.65, −0.36) (p=0.0096, 0.2328), CLP (−0.63, −0.23) (p=0.0110, 0.3233) compared with Q. Similar effects were seen for DMQ compared with DM and Q, except that DMQ demonstrated a small, but significant decrease in CIAT (−0.35, −0.34) (p=0.0357, 0.0254) at d15 and d29.

Conclusions: In ALS patients, DMQ had a significantly larger and more persistent effect on threshold, control and control of paroxysms in PBA manifested by crying than threshold, control, control of paroxysms manifested by laughing. A small, but persistent, effect by DMQ on control of intrusive amusing thoughts in PBA was demonstrated.

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P72 INCIDENCE OF COMMON ADVERSE EVENTS WITH AVP-923 IN THE TREATMENT OF INVOLUNTARY EMOTIONAL EXPRESSION DISORDER (IEED) PATIENTS OF DIFFERING AGE

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Background: IEED is associated with various neurological disorders and is characterized by uncontrollable episodes of laughing and/or crying out of proportion, or incongruent with underlying mood. IEED is also commonly known as emotional lability, pathological laughing and crying, emotional incontinence, and pseudobulbar affect. AVP-923 is an investigational therapy that has been previously shown to be safe and effective in the treatment of IEED in multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) patients, in two phase III clinical trials. A phase III open label safety trial including IEED patients with MS, ALS, traumatic brain injury, stroke, Parkinson's disease, dementias, and other associated conditions is currently ongoing.

Objective: Evaluate the safety profile of investigational therapy AVP-923 (capsules containing dextromethorphan hydrobromide (DM; 30 mg) and quinidine sulphate (Q; 30 mg), for the treatment of IEED in adults of varying age.

Methods: IEED patients were pooled from two phase III clinical trials, and an open-label safety study of AVP-923 (n=584). Incidence rates of common adverse events (AE) across all trials were analysed for predefined age groups of <45 (n=185), 45–60 (n=275), and >60 (n=124) years of age. All subjects that received study drug or control were included in the analysis.

Results: The observed incidence of common adverse events was similar in each defined age group. Common AEs included dizziness (18% <45 years, 19% 45–60 years, 20% >60 years, 9% placebo), nausea (28% <45 years, 21% 45–60 years, 26% >60 years, 15% placebo), and fatigue (23% <45 years, 18% 45–60 years, 13% >60 years, 20% placebo). There were no significant differences in AE rates across the defined age groups for those patients on AVP-923 vs. those on placebo.

Discussion and conclusions: AVP-923 common adverse event incidence rates were similar across all IEED age groups that were studied. The tolerability profile of investigational therapy AVP-923 is similar in

IEED patients of differing adult age groups. AVP-923 appears well tolerated in multiple studies of IEED patients with differing underlying diseases.

P73 THE COMBINATION OF DEXTROMETHORPHAN AND QUINIDINE IN THE TREATMENT OF LABILE ANGER AND FRUSTRATION IN PATIENTS WITH INVOLUNTARY EMOTIONAL EXPRESSION DISORDER (IEED)

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Background: IEED is a condition characterized by the disinhibition of emotional expression secondary to neurological disease or injury. Episodes typically involve involuntary affective displays such as laughing, crying, or related facial features that are incongruent or exaggerated in relation to underlying mood. IEED is associated with neurological diseases such as MS, ALS, Parkinson's disease, dementias; and neurological injuries, such as stroke and traumatic brain injury. Historical terms such as emotional lability, pathological laughing and crying, emotional incontinence, and pseudobulbar affect have also been used to describe the symptomatology of IEED. Dextramethorphan-quinidine (DM/Q) has been shown in two pivotal trials to reduce the frequency and intensity of episodes of crying and/or laughing associated with IEED, as measured by the validated Center for Neurologic Study-Lability Scale (CNS-LS). This study explored treatment effects of DM/Q on anger and frustration, compared to its effects on the hallmark symptoms of crying and/or laughing.

Objectives: Explore the effects of DM/Q (capsules containing dextromethorphan 30 mg and quinidine 75 mg) on labile affective displays of anger and frustration in patients with IEED.

Methods: Analysis of a double-blind, placebo-controlled, crossover study of patients with IEED was undertaken to determine the effect of DM/Q on labile anger and frustration. Patients were treated for four weeks with DM/Q and a similar time period with placebo, with a one-week washout period in between. A questionnaire consisting of 65 questions that focused on episodes of inappropriate tearfulness, laughter, anger, and frustration was administered at baseline and post-treatment. Responses were scored on a 5 point scale, with 1=applies never and 5=applies most of the time. All the questions, related to anger and frustration, were analysed for change from baseline along with the seven validated CNS-LS questions utilized in clinical trials of IEED.

Results: Patients taking DM/Q reported similar improvement in scores related to anger and frustration as the validated CNS-LS questions related to laughing and or crying. Patients reported 16% reduction from baseline in episodes of anger, 30% reduction in episodes of frustration, and a 29% reduction in the CNS-LS questions relating to laughing and/or crying.

Conclusions: DM/Q, previously shown to reduce crying and/or laughing associated with IEED, also appears to reduce anger and frustration. Further studies are needed to explore DM/Q effects on anger, frustration, and other affective disinhibition manifestations.

P74 LEVETIRACETAM TREATMENT FOR SPASTICITY IN MOTOR NEURON DISEASES: A CASE SERIES

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Background: Spasticity is a common problem in patients with motor neuron diseases (MNDs). There are two clinical types: tonic (producing muscle stiffness) and phasic (producing cramps and spasms). Together these can interrupt sleep, exacerbate fatigue, produce pain, impair balance and increase the risk of falls. The pathophysiology of spasticity in MNDs is poorly understood and no treatment is proven to improve it. A number of different agents are being used anecdotally (baclofen, tizanidine, benzodiazepines, dantrolen, gabapentin); side-effects from these include sedation, exacerbation of fatigue and weakness, and cognitive dysfunction. Levetiracetam (Keppra) is an anti-epileptic agent that may be useful in the treatment of spasticity. In support, levetiracetam reduces phasic (but not tonic) spasticity in patients with multiple sclerosis.

Objectives: To review a series of patients with MNDs treated with levetiracetam for spasticity.

Methods: We announced our hypotheses regarding levetiracetam and MNDs at the 2005 Northeast ALS Consortium Meeting. Since then, three ALS clinics (Duke University, Massachusetts General Hospital and the University of Utah) submitted case reports of patients with MNDs treated with levetiracetam for spasticity. We are compiling information about patient demographics, dosages and duration, and outcomes.

Results: A total of five patients make up the case series to date. Both ALS and PLS are represented, ages 37–67 years. Most of the patients have had inadequate responses to baclofen. Levetiracetam doses range from 250 to 3000 mg daily, exposures from one to three months. No serious side-effects have been reported. Three of the five patients report symptomatic improvement starting within 72 h of exposure to the medication. In some cases the symptomatic improvement is dramatic. One patient who had failed baclofen reported "being released from a vice". In some cases, there is also clear objective benefit – documentation of increased range of motion, improved ability to dress and lengthened walking distances.

Discussion: Given the unpredictable response to anti-spasticity agents currently being used in MNDs, this small case series suggests that levetiracetam warrants further controlled study. We currently have a Phase II open-label pilot trial underway that aims to assess safety and efficacy of levetiracetam in 20 patients with MNDs (ALS, PLS or PMA). Eligible patients have cramps with average severity 50/100 points, normal renal function and are on a stable riluzole dose, in addition to meeting other eligibility criteria. After a three-month baseline period, patients take levetiracetam at increasing doses up to 3000 mg per day over a nine-month period. 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. The results of this study should help determine whether levetiracetam is worth taking into a Phase III trial next year.

P75 BENEFICIAL EFFECTS OF INTRATHECAL IGF-1 ADMINISTRATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder caused by degeneration of the upper and lower motor neurons in the brain and spinal cord resulting in progressive paralysis of voluntary muscles. However, there is currently no effective pharmacological treatment for amyotrophic lateral sclerosis. In a transgenic mouse model of ALS, intrathecal infusion of insulin-like growth factor (IGF)-1 showed a promising increase in survival.

Objectives: To examine whether IGF-1 is effective for the prevention of disease progression, we performed a double-blind clinical trial to assess the effect of intrathecal administration of IGF-1 in patients with ALS.

Methods: Nine patients with ALS were randomly assigned to receive either a high dose (3 microg/kg of body weight) or low dose (0.5 microg/kg of body weight) of IGF-1 every two weeks for 40 weeks. The outcome measurements were the rate of decline of bulbar and limb functions (Norris scales) and forced vital capacity.

Results: The high-dose treatment slowed a decline of motor functions of the ALS patients in total Norris and limb Norris scales, but not in bulbar Norris or vital capacity. The intrathecal administration of IGF-1 had a modest but significant beneficial effect in ALS patients without any serious adverse effects.

Discussion and conclusions: Intrathecal IGF-1 treatment could provide an effective choice for ALS although further studies in more patients are needed to confirm its efficacy and optimize dosages of IGF-1.

P76 SAFETY AND PHARMACOKINETICS OF REPEATED DOSES OF TRO19622, A DRUG CANDIDATE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY

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Background: TRO19622 was selected for its ability to rescue rat motor neurons deprived of trophic factors. *In vivo*, TRO19622 increases survival, maintains weight and motor function in SOD1G93A mice (3 and 30 mg/kg/day) and increases survival in a mouse model of SMA (NSE-Cre; F7/F7 – Judith Melki) (30 mg/kg/day, sc). The molecule is orally bioavailable, crosses the blood-brain barrier and has a large safety margin (NOAEL of 1 g/k/day in four-week toxicity testing in rat and dog).

Objective: To assess the safety and determine the pharmacokinetic (PK) profile of multiple oral doses of TRO19622 in healthy subjects.

Design: Four oral doses of TRO19622 (50, 150, 250 and 500 mg) were administered for 11 consecutive days in four groups of 12 healthy male Caucasian subjects (nine on TRO19622, three on placebo) in a double-blind, randomized, sequential, multiple-dose escalation trial.

Results:
Safety: No SAE as well as no major issues in safety assessment were reported. Sixty-nine Treatment Emergent Adverse Events (TEAE) were reported: 18 were considered possibly related to the investigated medicinal product (IMP), 22 unlikely related and 27 were considered unrelated to the IMP. Among the possibly related TEAEs, seven occurred on placebo; two on TRO 19622 50 mg dose; none on TRO 19622 150 mg; two on 250 mg dose and seven on TRO 19622 500 mg dose. These TEAEs were mild (48) or moderate (21) in intensity. None was rated as severe. The most common TEAEs ($n \geqslant 4$ episodes) were diarrhoea (nine episodes), headache (seven episodes), constipation (four episodes), pharyngitis (four episodes) and back pain (four episodes). There was no correlation between occurrence of TEAEs and dosage. No relevant change in vital signs, ECGs parameters, laboratory tests or physical examinations were observed after repeated administration.

Pharmacokinetics. TRO19622 absorption and elimination were slow after oral dosing whatever the dosage: t_{max} of about 10 h and TRO19622 concentrations measurable up to 19 days after dosing. Mean terminal elimination half-life was about 120 h and comparable between doses. On day 1 when the dose increased in a ratio of 3, 5 and 10 (from 50

to 150, 250 and 500 mg, respectively), C_{max} increased in a ratio of 2.2, 4.4 and 10.2 and AUC_{0-t} increased in a ratio of 2.1, 4.6 and 10.8. At steady-state, reached on day 11 whatever the group, C_{max} increased with dose in a ratio of 2.1, 7.2 and 12.2 and $AUC_{0-\tau}$ increased in a ratio of 2.0, 6.5 and 11.6. Mean accumulation ratios of C_{max} and C_{trough} observed between day 1 and day 11 were about 4. Plasma PK profiles were similar between subjects and across doses, the coefficient of variation of C_{max} and $AUC_{0-\tau}$ were between 21 and 47% on day 11.

Conclusion: TRO19622 repeated administration up to 500 mg per day was well tolerated in healthy volunteers. PK results suggest that target effective concentrations derived from *in vitro* and several *in vivo* pre-clinical models can be reached at a QD dose of 250 mg and above.

Acknowledgement: The study was supported by Association Française contre les Myopathies.

P77 A MULTICENTRE, DOSE RANGING SAFETY AND PHARMACOKINETICS STUDY OF ARIMOCLOMOL IN ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a rare motor neuron disease, usually fatal within three to five years. Although the cause of the disease is unknown, the presence of characteristic protein aggregates in relevant cell types implicates a role for protein misfolding in the disease. Arimoclomol is a small molecule that up-regulates heat shock proteins (HSP) in cells. HSPs function as protein-folding chaperones and are critical components of a cell's response to stress. Arimoclomol extends survival when given both pre-symptomatically and at disease onset in a mutant superoxide dismutase (SOD1) transgenic mouse model of ALS (1). In treated mice, arimoclomol delayed the death of motor neurons and the associated loss of motor unit potentials. It has also been demonstrated to have neuroprotective and neuroregenerative effects in acute models of nerve injury. Therapeutic agents that target protein misfolding, such as arimoclomol, may be helpful in ALS.

Objectives: The primary objective of this study was to assess the safety and tolerability of arimoclomol compared with placebo over 12 weeks of treatment in patients with ALS. The secondary objective of this study was to determine the pharmacokinetic characterization of arimoclomol in blood and cerebrospinal fluid (CSF) penetration in 40 of the 84 total subjects.

Methods: A double-blind, placebo-controlled, clinical trial is being conducted. Eighty-four research subjects with ALS have been randomized to receive oral arimoclomol at one of three dosages (75, 150, and 300 mg/day) or placebo for 12 weeks. Visits and safety testing occur every two weeks. Forty research participants have blood drawn to determine arimoclomol and riluzole levels at the following time points: prior to receiving study dose, and at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h after receiving the study dose at the baseline and week 4 study visits. To determine the degree to which arimoclomol enters the CSF, samples are taken by standard lumbar puncture at the week 4 visit in these 40 participants.

Results: Enrollment was completed in March 2006 and the last study visit is expected in July 2006. The full study report will be available by September 2006. All pharmacokinetics visits are complete.

Discussion and conclusions: Should arimoclomol show favorable 12-week tolerability and safety in patients with ALS, the data from the study would be used to support an efficacy study in ALS. The safety and the pharmacokinetic analyses data will guide selection of dosage for a subsequent efficacy study.

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P78 REPEATED ADMINISTRATION OF EDARAVONE, A FREE RADICAL SCAVENGER, FOR PATIENTS WITH ALS

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Background: Oxidative stress is presumed to contribute to pathogenesis of ALS.

Objectives: We investigated the efficacy and safety of edaravone, a free radical scavenger previously approved for acute cerebral infarction, in ALS patients.

Methods: Within an open trial design, 20 subjects with ALS received either 30 mg (five subjects) or 60 mg (15

subjects) of edaravone via intravenous drip once per day. Two weeks of administration were followed by a two-week observation period. This series was repeated six times. A change in the revised ALS functional rating scale (ALSFRS-R) was used as the primary endpoint, while secondary endpoints of respiratory function (%FVC), and 3-nitrotyrosine (3NT) levels, a marker of oxidative stress, were assessed.

Results: Efficacy was evaluated in the 60 mg group of patients. Six months following administration, the ALSFRS-R was inhibited by 2.4 ± 3.5 points (paired *t*-test, $p=0.035$) compared to the six-month prior level. The change in %FVC at six months following administration was $-4.47 \pm 12.30\%$. In almost all patients, CSF 3NT was markedly reduced to almost undetectable levels at the end of the six-month treatment period. Stratified analysis showed that in the group pf patients with equal to or greater than 41 points ALSFRS-R at the commencement of the study ($n=4$), %FVC was increased by 7.23 ± 14.77, while in patients with less than 41 points ALSFRS-R ($n=8$), %FVC was reduced by 10.31 ± 5.19.

Conclusion: In the previous placebo-controlled short term study, efficacy of edaravone was also demonstrated in %FVC and in the change of ALSFRS-R score in patients with equal to or greater than 41 points ALSFRS-R. Combined with the present six months open study, edaravone might inhibit the progression of ALS in the early stage. A confirmative placebo-controlled long-term study is currently under progress in Japan.

P79 SAFETY CONCERNS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS WHO ARE ON RILUZOLE AND BU NAO GAO (BNG) CHINESE HERBAL MEDICATION

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Background: BNG, in Chinese, 'tincture or solution to revive the brain', contains 14 herbs: Radix angelica sinensis (*DangGui*) 29.3%, Ligusticum chuanxiong (*ChuanXiong*) 7.3%, Hirudo (*ShuiZhi*) 3.7%, Astragalus membranaceus (*HuangQi*) 11%, Paeonia rubrae (*ChiShao*) 7.3%, Lycium chinense mill (*GouQiZi*) 4.4%, Polygonatum sibiricum (*HuangJing*) 7.3%, Curculigo orchioides (*XianMao*) 3.3%, Epimedium grandiflorum (*YinYangHuo*) 3.3%, Plastrum testudinis (*ShengGuiBan*) 5.5%, Cornus officinalis (*ShanZhuYu*) 3.7%, Psoralea corylifolia (*BuGuZhi*) 4.4%, Leonurus heterophyllus (*YiMuCao*) 7.3%, Glycyrrhiza uralensis (*Gancao*) 2.2%. Western literature has no clinical descriptions of observed side effects of BNG alone or in combination with riluzole.

Objective: To report observations of subacute onset of weakness or rapid deteroration of ALS when riluzole and BNG treatments were combined.

Methods: Observational study in patients who experienced ALS worsening following subacute weakness on reinstitution of riluzole when it had been stopped for weakness in combination therapy with BNG.

Results: Four arm onset ALS patients, three males and one female, age 55–62 years, were self-treated with BNG either before ($n=2$) or after ($n=2$) initiation of riluzole treatment. On combination therapy, these patients noted asthenia, weakness, loss of appetite, weight loss (10–22 pounds) and decreased ALS FRS-R. Two patients rechallenged with riluzole worsened and discontinued this medication. One of these patients was rechallenged twice with development of weakness and asthenia each time. One patient added BNG to riluzole with consequential weakness that improved with cessation but returned when rechallenged with BNG. Deep vein thrombosis developed in one of four patients on BNG.

Discussion and conclusions: BNG, a poorly characterized concoction of multiple herbs may be associated with subacute onset of asthenia, worsening of ALS clinimetrics, anorexia and weight loss. ALS patients on riluzole may be at risk when starting BNG. A rapid change in clinical status in an ALS patient should lead to investigations of combination therapy with alternative medicines, particularly BNG.

P80 COMBINATION THERAPY WITH MESENCHYMAL STEM CELL AND ERYTHROPOIETIN IN PATIENTS WITH ALS

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Background: The devastating nature of amyotrophic lateral sclerosis (ALS) has led to a major effort to find a treatment capable of arresting the disease. To date, in spite of more than 100 trials in ALS, it has been shown that only one medication, riluzole, has a marginal effect on survival and no effect on muscle strength, quality of life or functional capacity. Although stem cell therapies, such as human umbilical cord cells and adult autologous stem cells, have been tried on a small scale in ALS patients, no positive results have as yet been reported with monotherapy. However, in previous reports, erythropoietin has proved its neuroprotective and regenerative properties in CNS injury.

Objectives: To evaluate the effects of the combination therapy with mesenchymal stem cells and erythropoietin in patients with ALS.

Methods: Eight patients with definite ALS were recruited and treated. Autologous mesenchymal stem cell collection was performed by *ex vivo* expansion of mesenchymal stem cells following bone marrow aspiration from the patient's

own posterior superior iliac spine. Erythropoietin was injected intravenously one day before stem cell therapy. Stem cells were infused intrathecally over a period of minutes using a spinal needle at the level of L3/4. The same procedure was done twice at intervals of 35 days. The patients were monitored by clinical evaluation including forced vital capacity, ALSFRS-R, Norris score, and Appel score. The neurophysiological index was used as a neurophysiologic assessment.

Results: At the second day after stem cell injection, a mild increase in muscle strength was observed in six patients, and improvement of spasticity was shown in five patients. In addition, two patients experienced improvement in speech and swallowing, and no further functional deficit was observed in five patients during the first two months after first treatment. At the ninth month after the first treatment course, functional decline analyses of ALSFRS-R showed a less steep slope after treatment. No patient manifested severe adverse events such as death or respiratory insufficiency. Post-puncture headache, which was transient and disappeared spontaneously, was reported in three patients.

Conclusions: We suggest that this approach including mesenchymal stem cell and erythropoietin has potential therapeutic efficacy and is worthy of further investigation.

P81 ASSESSMENT OF METHODS TO REDUCE MISSING DATA FROM ALS TRIALS

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Background: One of the great barriers facing ALS researchers is how best to reduce patient dropout and missing data from clinical trials. Missing data reduce the power of the study and can lead to inconclusive or biased results. Full patient compliance coupled with easy to administer outcome measures is essential for providing high quality data at analysis. Open and frequent dialogue with patients both before enrolling and during the trial might help reduce dropout. It is not known which outcome measures best reduce missing data.

Objective: To describe interventions to reduce dropout rates in a phase II clinical trial, and to determine which clinical outcome measures are associated with the least quantity of missing data.

Methods: We conducted a randomized controlled trial in 30 patients with one baseline and six monthly visits, giving a total of 210 possible patient visits, and assessed the standard outcome measures for data quality. Of the 30 participants, four patients died prior to completion, leaving 13 visits uncompleted and 197 visits for analysis. The ALS Functional Rating Scale-Revised (ALSFRS-R), Quality of Life (QOL) questionnaire, manual muscle testing (MMT), and forced vital capacity (FVC) were performed at each clinic visit. Telephone contacts were made when patients could not come to the clinic. At those times, FVC and MMT were not performed. The investigator, coordinator and physical therapist met with each patient individually at each clinic visit. Telephone contacts, for those who could not attend clinic due to weakness, were performed by the coordinator and physical therapist.

Results: There were no dropouts for reasons other than death. Of a possible 197 visits, only one patient missed a full visit. ALSFRS-R and QOL survey were completed 196 times (99.5% complete data). MMT evaluations were collected on 190 visits (96%). Positions that required patients to lie either prone (75%) or supine (88%) had a higher percentage of missing data than positions in which the patient could sit upright (97%). FVC was performed 187 times (95%) overall. Every patient who performed at least one pulmonary function test, successfully completed three attempts.

Conclusions: Investment of time by the research team was an effective means of reducing patient dropout. Management of clinical trials, therefore, should include a high level of involvement from the investigator and study clinicians beginning with the pre-screening process and continuing through all study visits. Regular discussions about research and emphasis on good clinical care may help promote retention in clinical trials. Additionally, the use of easily administered outcome measures can further reduce the amount of missing data for analysis. Measures that do not require patients to lie down and that can be administered by phone, such as the ALSFRS-R, best reduce the amount of missing data.

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P82 UNIFIED ELECTRONIC DATA CAPTURE AND DATA MANAGEMENT SYSTEM FOR CLINICAL TRIALS AND BIOMARKER STUDIES IN ALS

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Background: Sharing research data from multiple biomarker studies and clinical trials in amyotrophic lateral sclerosis (ALS) is challenging as different centres use proprietary data storage formats and data dictionaries. Conduct of multiple trials in ALS highlights a growing need for standardization of common data elements (CDE) for the exchange, sharing, integration, analyses, storage and retrieval of data. One of the proposed solutions is creation of the Unified Electronic Data Capture and Data Management system to be used by the entire ALS research community in the US and Canada.

Objectives: Create specifications and develop a single web-based system for data capture and management to be used by the members of the American ALS Research

Group (ALSRG) whose mission is to 'foster communication, collaboration and research in North America into the cause and treatment of ALS'. Future international collaboration is also feasible.

Methods: With more ALS clinical trials and biomarker studies being conducted by multiple research organizations and individual researchers, there is a clear need to create standards in data acquisition and management. As the collaboration at the methodological and organizational levels proved to be successful with the ALS Biomaterial and Data Banking initiative's commencement in 2006, a larger effort is needed in definition of standard ALS-specific CDE and requirements specifications for the unified web-based system. The design of such a system will require flexible architecture to be able to create new studies utilizing library CDE as building blocks. The system will also allow association of new visits and forms with the existing subjects. This feature is necessary when an additional study is being conducted on a subset of subjects participating in the initial study.

Results: Standards for ALS-specific CDE are being created. Along with this effort, the system specifications must be developed with input from all interested parties. The unified system will be deployed on a central server and securely accessible to all ALSRG members.

Conclusions: The following are the recommendations to the ALS research community for successful specification, design and deployment of the Unified Data Capture and Data Management system in ALS:

- Form work groups to develop proposals for the unified system
- Form CDE committee to specify ALS-specific CDE such as outcome measures, disease case report forms (CRFs) and procedures
- Develop processes for approval and submission of CDE to the system library of forms
- Create specification to the unified system based on the PharmaENGINE™ platform that is already deployed by the NEALS consortium and Neurology Clinical Trials Unit of Massachusetts General Hospital
- Develop requirements to the system that will allow maximum flexibility in CDE CRFs creation and utilization through user interface
- Define responsibilities of the data management team
- Develop compliance to regulatory documents strategies
- Find a sponsor to underwrite the effort

P83 THE TIMED GET UP AND GO TEST, A FUNCTIONAL OUTCOME MEASURE FOR CLINICAL TRIALS IN ALS

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Background: Clinical outcome measures are used as surrogates for survival in clinical trials to reduce sample size and treatment duration. Ideally, outcome measures provide meaningful functional assessments, are easily administered, can be performed in various settings, require minimal equipment or training, and do not burden patients. The Timed Get Up and Go (TGUG) test measures the time it takes an individual to stand up from a chair, walk three meters, turn around and sit down in the same chair. TGUG is used to assess functionally meaningful mobility. It is a reliable test for quantifying mobility in the elderly and is a sensitive measure for identifying elderly individuals prone to falls. In Parkinson's disease it correlates with Unified Parkinson Disease Rating Scale scores, is sensitive to changes associated with levodopa use and correlates with and predicts fatigue.

Objectives: To assess the performance of TGUG in a phase II clinical trial in ALS.

Methods: We conducted a six-month, prospective, randomized phase II trial of glatiramer acetate in ALS. Thirty subjects were assigned to one of two treatment groups, or a control group, and underwent monthly TGUG, ALS Functional Rating Scale Revised (ALSFRS-R), forced vital capacity (FVC), manual muscle testing (MMT) and quality of life (QOL) assessments. Pearson correlation coefficients were determined for baseline scores and slopes. *T*-tests were used to compare differences in means between those who could and could not perform TGUG. Survival was assessed using Kaplan-Meier and Cox proportional-hazards methods.

Results: There were no differences in outcomes between treatment groups, which were therefore combined ($n=30$) for analysis. Analyses included imputed values for those unable to perform TGUG ($n=9$). The mean TGUG time was 19.3 s (range 6.62–73.8). TGUG time at baseline strongly correlated with leg MMT ($r=-0.67$, $p<0.0001$), proximal leg MMT ($r=-0.75$, $p<0.0001$), total MMT ($r=-0.56$, $p=0.0014$), and with ALSFRS-R motor ($r=-0.47$, $p=0.03$) and gait subscores ($r=-0.41$, $p=0.009$). ALSFRS-R motor ($p=0.0019$) and leg ($p=0.0047$) subscores, proximal leg MMT ($p<0.0001$), distal leg MMT ($p=0.0352$), and total MMT ($p=0.0043$) scores were significantly different in those who could and could not perform TGUG at baseline. The slope of TGUG correlated with the slopes of QOL ($r=0.62$, $p=0.0003$) and proximal leg MMT ($r=0.42$, $p=0.03$), and survival was found to be better for those who performed TGUG above the median than for those who performed below the median ($p=0.0561$).

Conclusion: TGUG correlates with standard outcome measures, which are different in those who can and cannot perform the test, and may predict survival. TGUG could be a useful addition to the ALSFRS-R as a simple, less expensive, sensitive, and clinically meaningful outcome in trials of ALS.

P84 PERFORMANCE OF IN-PERSON VS. PHONE ALSFRS-R IN A RANDOMIZED, CONTROLLED PHASE III CLINICAL TRIAL OF MINOCYCLINE IN ALS

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Background: The ALSFRS-R has become a principal clinical outcome measure for trials in ALS. It has good reliability, internal consistency, and content validity: declines in tandem with pulmonary function and muscle strength; and predicts survival more strongly than other clinical measures. Telephone administration may be an important way to reduce missing data in ALS trials. Standardized procedures are now used for in-person and phone administration.

Objective: To assess differences in phone and in-person ALSFRS-R scores in a phase III clinical trial that uses the ALSFRS-R as the primary outcome measure.

Methods: Monthly scores were obtained from 471 subjects in an ongoing phase III clinical trial of minocycline in ALS. One hundred and forty subjects had eight or more monthly scores and at least one evaluation obtained by phone. The number of phone assessments ranged from 1 to 11 with 55 subjects having more than one phone assessment. A locally weighted least squares (lowess) curve was fitted to each subject's data ignoring whether the score was from the clinic or by phone. For each data point a residual, equal to the difference between the observed score and the lowess fit was calculated. The distribution of residuals was compared by assessment type (clinic vs. phone) and a two-sample *t*-test was used to test for differences between types of administration. Analysis of variance was performed to determine whether visit number or total numbers of clinic and phone assessments affected the fits.

Results: The deviations of the phone scores from the lowess fits are similar to those for the clinic scores. The 1302 residuals for clinic (mean 0.08; SD=1.41) scores are not different from the 346 residuals for phone (mean=−0.06; SD=1.46) scores (*t*-statistic=1.59, 2-sided $p=0.11$). There are no differences in residuals by visit number (e.g. fifth visit), total number of visits, numbers of clinic visits or numbers of phone visits.

Conclusions: There is no difference between phone and in-person ALSFRS-R scores in the large phase III clinical trial where a standard format was used to obtain data. The use of ALSFRS-R as a primary outcome measure is further strengthened because of its ability to produce valid and complete data from those patients too weak to travel to a research clinic.

P85 ORAL ADMINISTRATION OF MEMANTINE PROLONGS SURVIVAL IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background and objectives: N-methyl-D-aspartate (NMDA)-mediated neurotoxicity and oxidative stress have been implicated in the aetiology of motor neuron disease. Memantine is a low- to moderate-affinity NMDA receptor antagonist and it may have a potential to protect against motor neuron degeneration.

Methods: Thirty transgenic mice expressing the G93A SOD1 mutation were randomly divided into control, low dose (30 mg/kg), and high dose (90 mg/kg) memantine groups, and mematine was supplied daily in the drinking water, beginning at 75 days of age. Body weight, survival, and behavioural performance including rotarod test, paw grip endurance (PaGE), and hindlimb extension reflex were assessed in control and memantine diet groups.

Results: Clinical disease was evident in the G93A transgenic mice by 11 weeks of age. Memantine was tolerated well. However, although statistically not significant, its treatment showed a tendency toward a protective effect on rotarod performance or on hindlimb extension reflex. Moreover, the low dose memantine group significantly prolonged survival of transgenic mice compared with controls (141 days vs. 134 days, $p<0.05$).

Conclusions: These findings suggest that memantine, even if administrated at symptom onset, may have some beneficial effect on patients with amyotrophic lateral sclerosis (ALS).

P86 NEUROPROTECTIVE EFFECTS OF TACROLIMUS ON THREE MODELS OF SPINAL MOTONEURON DAMAGE

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Background: Tacrolimus, a neuroimmunophilin ligand, possesses pleiofunctional effects on several kinds of neurons.

Objectives: The purpose of this study is to examine whether this drug can expand survival of motor neurons in

three experimental models of damaged spinal motor neurons.

Methods: *Experiment 1*. Organotypic spinal cord cultures were made from 10-day-old Sprague-Dawley rats. Cultures were exposed to glutamate (10^{-5} M) or glutamate (10^{-5} M) + tacrolimus (10^{-7} to 10^{-5} M) for 14 days. The number of motor neurons (size$\geqslant$25 μm) and choline acetyltransferase activity (ChAT) were determined in the non-treated control, the glutamate and the glutamate + tacrolimus groups (n=20 slices/group).

Experiment 2. The left sciatic nerve was cut near the obturator tendon in one-day-old Sprague-Dawley rats. After injury, rats received intraperitoneal injection of tacrolimus (0.1, 1.0, 5.0 mg/kg) or vehicle daily for 14 days. The number of large sized motor neurons ($\geqslant$25 μm) with prominent nucleoli was counted in both sides and the survival of motor neurons (%) was calculated as the number of motor neurons in the injured side /that of non-injured $\times$100.

Experiment 3. Wobbler mice were given an intraperitoneal injection of tacrolimus (1.0, 5.0 mg/kg) or vehicle from disease onset (three to four weeks of age) daily for four weeks. Motor symptoms of the forelimbs were evaluated every week. After the treatment, neuropathological changes of the biceps muscles and the C5–6 cord were compared among tacrolimus- and vehicle-treated wobbler mice (n=10 mice/group).

Results:
Experiment 1. Compared to control culture, the survival of motor neurons and the ChAT activities were decreased by 30–40% in glutamate-exposed culture. Three doses of tacrolimus-treated cultures significantly inhibited neuronal death ($p<0.05$) and loss of ChAT activity ($p<0.05$).

Experiment 2. After axotomy, the survival of motor neurons was approximately 50% in vehicle-treated rats. High dosage tacrolimus (5 mg/kg) treatment significantly saved axotomized motor neurons by 20% ($p<0.05$) compared with vehicle.

Experiment 3. Tacrolimus treatment did not delay the progression of motor deficits, denervation muscle atrophy and spinal motor neuron degeneration.

Discussion and conclusions: We studied the neuroprotective effects of tacrolimus on spinal motor neurons in *in vivo* and *in vitro* experimental models. This drug has no clinical effects in mutant superoxide dismutase (G93A)-transgenic mice (1). The present and previous studies indicate that tacrolimus treatment protects spinal motor neurons against acute models of glutamate-neurotoxicity and axotomy but has no benefits for chronic disease models of wobbler mice and mutant superoxide dismutase-transgenic mice.

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P87 CHIP OVEREXPRESSION REDUCES THE MUTANT AR PROTEIN AND AMELIORATES PHENOTYPES OF THE SBMA TRANSGENIC MOUSE MODEL

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Background: Spinal and bulbar muscular atrophy (SBMA) is an inherited motor neuron disease caused by the expansion of the polyglutamine (polyQ) tract within the androgen receptor (AR). The pathologic features of SBMA are motor neuron loss in the spinal cord and brainstem, and diffuse nuclear accumulation and nuclear inclusions of the mutant AR in the residual motor neurons and certain visceral organs. Molecular chaperones are sequestered to inclusions, suggesting that molecular chaperones may be actively engaged in an attempt to degrade or refold components of the inclusions. CHIP (carboxyl terminus of Hsc70-interacting protein) has three tetratrico peptide repeat (TPR) domains, which interact with the molecular chaperones Hsp70 and Hsp90, and a U-box domain that interacts with the proteasome and confers E3 ubiquitin ligase activity on CHIP. CHIP has been shown to interact with ubiquitinated unfolded proteins trapped by molecular chaperones and degrade them, thus acting as a 'quality control E3'.

Objectives: CHIP overexpression has been shown to suppress inclusion formation and cellular toxicity in cellular and zebrafish polyQ disease models. In this study, we examine the effects of CHIP overexpression on a cultured cell model and the transgenic mouse model of SBMA to explore a potential strategy for SBMA therapy.

Methods: The full-length human CHIP fragment was subcloned into pCAGGS vector. The final plasmids were digested to remove the transgene. We generated CHIP overexpression mice by microinjection into BDF1 fertilized eggs. We crossed the mice expressing full-length human AR with 24 or 97-polyQ tract (AR-97Q mice, 7–8 line) with CHIP overexpression mice.

Results: We demonstrated in a neuronal cell model that transient overexpression of CHIP reduced the monomeric mutant AR more than the wild-type, suggesting that the mutant AR is more sensitive to CHIP than is the wild-type. We also demonstrated high expression of CHIP inhibited neuronal nuclear accumulation of the mutant AR and ameliorated motor impairments in the SBMA transgenic mouse model. Western blot analysis showed that both the high-molecular-weight form of mutant AR protein complexes retained in the stacking gel and a band of monomeric mutant AR monomer in the spinal cord and muscle of the transgenic mice were diminished in the double transgenic mouse.

Discussion and conclusions: We reported that overexpression of CHIP markedly ameliorated clinical and

pathological phenotypes, and that this amelioration was correlated with the reduction of mutant AR protein complexes in the mouse model of SBMA. Furthermore, the amount of monomeric mutant AR was also significantly reduced in the double transgenic mice, suggesting that degradation of mutant AR may have been accelerated by overexpression of CHIP. Thus, CHIP overexpression would provide a potential therapeutic avenue for SBMA.

P88 OLFACTORY ENSHEATHING CELL TRANSPLANTATION IN ALS MODEL MICE

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Background: An effective treatment has still not been established for amyotrophic lateral sclerosis (ALS). In recent years, ever-greater hopes have been placed in the ability of stem cells to cure a damaged or degenerated central nervous system (CNS). However, motor neurons are widely distributed throughout the CNS. For the treatment of ALS, replenishing the stem cells or the alternatives by focal injection would be impractical for clinical application. To overcome this problem, we developed a transplantation method for mice that allows even diffusion of donor cells throughout the CNS.

Objectives: We chose the olfactory ensheathing cell (OEC) as a suitable donor cell. The OEC reportedly promotes regeneration and remyelination of injured motor pathways and enhances motor function recovery in experimental animal models. As a recipient we used transgenic mice with the mutated Cu/Zn superoxide dismutase (SOD1) gene (ALS-TgM) (1). First we confirmed the safety of the transplantation procedure, then we transplanted OECs via the fourth ventricle in ALS-TgM.

Methods: The OECs were extracted from mice which ubiquitously expressed green fluorescent protein (GFP), and then purified and expanded. The transplantation was performed on 90-day-old mice (wild-type mice and ALS-TgM) according to the report for rats (2), albeit with major modifications. Beginning one week prior to the operation, weekly clinical evaluations were made of body weight, hind limb extension score, and footprints, up to 200 days for wild-type and until death for ALS-TgM. Pathological observations were also performed with immunofluorescent staining in frozen sections.

Results: There were no differences between the OEC transplanted and non-transplanted wild-type mice with regard to perioperative side-effects as well as long-term motor functions. With regard to ALS-TgM, after clinical evaluation, no significant differences could be found between OEC-treated and non-treated ALS-TgM groups, nor were any differences noted with regard to age at onset, age at death, or disease duration. In the pathological observation, OEC survival in spinal cord was confirmed as long as 30 days after the transplantation.

Discussion and conclusions: We have established a novel cell transplantation method that can be used on mice. In the present experiment, however, we were unable to detect any beneficial effects of OEC transplantation on ALS-TgM. Considering that OECs survive about 30 days in the recipient spinal cord, modulation of the appropriate transplant time would appear necessary. Furthermore, since it has previously been reported that neural or mesenchymal stem cells have shown promising results in the treatment of ALS, it would seem sensible to examine stem cells other than OEC in greater detail.

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P89 TOWARDS GENE THERAPY USING GENETICALLY ENGINEERED CELLS AND STEM CELLS IN A MOUSE MODEL OF ALS

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Background: We have shown olfactory stem cells (OSCs) to be multipotent (1). Olfactory ensheathing cells (OECs) have been therapeutic in a rat model of paraplegia (2). These cells are a glial type and are intrinsically supportive to neurons. They surround the olfactory nerve and assist its continual regeneration. Both these cell types have been labelled with reporter genes under constitutive and tissue-specific promoters introduced via retroviral vectors enabling stable integration. This result suggests potential to undertake gene therapy using these cells. Watanabe has created a mouse model of ALS by introducing a mutation to SOD1 (3). These mice mimic the symptoms and disease progression seen in human ALS patients, therefore allowing behavioural assessment and histological analysis of any potential gene therapy and cell transfer strategies.

Objectives: We propose to replace damaged motor neurons with the seeding of neural stem cells and protect and nurture new and damaged neurons with olfactory ensheathing glia expressing genetically introduced protective factors.

Initial aims. 1) To generate lentiviral constructs expressing GDNF and BDNF under various tissue specific promoters, a constitutive promoter and an inducible promoter. 2) To transduce these viral constructs into Rosa-GFP OECs and OSCs. 3)To test these cells *in vitro* for genuine delivery of growth factors. 4) To establish cell transplant delivery and detection in SOD1 mutant mice. 5) To test various combinations of OEC-growth factor delivery cells and stem cells in these mice. 6) To assess behavioural

parameters to indicate any reduction in disease progression. 7) To analyse post-mortem tissue.

Methods: Gene sequences were obtained by PCR from genomic and cDNA sources and assembled in commercial plasmids. Inserts were confirmed using restriction analysis and sequencing. Lentivirus particles were generated by transfection in Virapower 293FT producer cell line and components of the Virapower Lentiviral Expression System (Invitrogen). OECs from Rosa-GFP (BL 6 background) were transduced with various lentiviral vectors, propagated *in vitro* and assayed for production of GDNF using antibody detection. OSCs from these mice were propagated as described and in some cases transduced with viral vectors. Methods of grafting were initiated and OECs were transplanted into the fourth ventricle along the midline 6 mm backward from Bregma suture at 3.75 mm in depth. Immunosuppression utilized cyclosporin (Cs)10 mg/kg/day, i.p. and tacrolimus (FK506) 3.2 mg/kg/day, p.o. dissolved in drinking water. Transplanted OECs were detected using fluorescence microscopy for GFP signal.

Results: To date the constructs CMV-GDNF, NF-GDNF, GFAP-GDNF and Tet-CMV-GDNF have been constructed, packaged and transduced into target cells. *In vitro* expression of GDNF has been assessed. Transplantation of GFP-labelled OECs has been successful with detection 30 days after transplant.

Conclusion: Feasibility for this strategy has been demonstrated.

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P90 HUMAN NEURAL PROGENITOR CELLS SECRETING GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) PROTECT LARGE MOTOR NEURONS IN THE SPINAL CORD OF ALS RATS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease causing the progressive loss of brain and spinal cord motor neurons. Mutations in the superoxide dismutase 1 (SOD1) gene, which are associated with 1–2% of human patient cases, are thought to induce ALS in a gain of function effect through an unknown pathway suggested to include protein accumulation and aberrant enzymatic activity leading to increased reactive oxygen/nitrogen species. Recent studies suggested the involvement of neuronal-glial interactions in ALS pathogenesis (1). Motor neuron degeneration in ALS may be due to dysfunction in the surrounding astrocyte populations.

For both drug delivery and astrocyte replacement, human neural progenitor/stem cells (hNPC) can be used as these cells can migrate and integrate into the damaged nervous system (2). Furthermore, recent studies have shown that in animal models of ALS, growth factors such as glial cell line-derived neurotrophic factor (GDNF) can have significant neuroprotective effects. As stem cells migrate and integrate into the damaged nervous system, produce astrocytes and can be genetically modified to release these growth factors, they represent an ideal source of tissue for delivery. Most recently we prepared hNPC from developing cortex secreting glial cell line-derived growth factor (hNPC-GDNF) and transplanted these cells into the lumber spinal cord of rats overexpressing the G93A SOD1 mutation (SOD^{G93A}) and observed cell survival, migration, and maintenance of GDNF release in the end-stage disease animal (3).

In the current study, we showed that hNPC-GDNF could survive in the spinal cord of ALS rats without tumour formation and spread up to 6.7 mm from the injection sites. We also confirmed high expression (up to 820 pg/mg tissue protein) of GDNF in the transplanted area using ELISA even at the disease end-stage. To determine whether hNPC-GDNF transplantation affected motor neuron number and cell size, hNPC-GDNF was unilaterally transplanted into the lumbar spinal cord of pre-symptomatic ALS rats (70 days of age). The spinal cords were collected at the mid-stage of disease (six weeks after surgery). There was a significant protection of large motor neurons ($>700\ \mu m^2$) in the region of GDNF release compared to the non-grafted side.

These results indicate that hNPC-GDNF can survive, maintain GDNF release, and protect motor neuron loss at the mid-stage of disease in a rat model of ALS.

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P91 MUSCLE IS NOT A PRIMARY TARGET IN ALS AS REVEALED BY GENE THERAPY IN MUSCLE OF ALS MICE

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal, progressive paralysis arising from premature death of motor neurons. An inherited form is caused by dominant mutation in the ubiquitously expressed enzyme superoxide dismutase (SOD1), whose non-cell autonomous toxicity to motor neurons requires mutant mediated damage to as yet undefined non-neuronal cell types.

Objective: Given the severe denervation induced muscle atrophy, two gene therapy approaches were tested in $SOD1^{G93A}$ mice to evaluate the role of muscle in ALS.

Methods: To directly assess whether increased muscle proliferation affected disease course in a mouse model of inherited ALS caused by mutation in SOD1, AAV1-follistatin or AAV-GFP (1×10^{10} viral genomes per injection) was injected bilaterally via intramuscular delivery into the hindlimb quadriceps and tibialis anterior muscles of sixteen 40-day-old (equal distribution of male and female) animals. To determine the extent of hyperplasia, hypertrophy and/or muscle sparing in the ALS animals, myofiber numbers within the gastrocnemius muscle were counted in serial sections of AAV-follistatin- or AAV-GFP- treated groups ($n=8$ animals).

To test whether $SOD1^{G93A}$ damage directly within muscle contributes to toxicity, a lentivirus was constructed that encodes an siRNA directed against SOD1. Since lentivirus pseudotyped with VSV-G is not retrogradely transported, intramuscular injection of this virus produced siRNA exclusively within the muscles.

Results: Muscle mass was enhanced with AAV encoded expression in muscle of follistatin, a potent stimulant of muscle growth. Despite increase in muscle mass, myofiber number, fiber diameter and overall strength, survival was not significantly affected. Suppression of mutant SOD1 accumulation by viral delivery to muscles of transcription mediated short interfering RNA against SOD1 did not maintain grip strength. Thus, in $SOD1^{G93A}$ mice mutant damage to muscles is not a significant contributor to pathogenesis in ALS.

Discussion: These data demonstrate that muscle is not a significant contributor to toxicity in ALS mice. While muscle is not a direct therapeutic target, it may nevertheless be an attractive target for synthesis of factors, such as IGF-1, whose delivery to spinal motor neurons can enhance motor neuron survival.

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THEME 6 *IN VIVO* EXPERIMENTAL MODELS

P92 PROLIFERATION OF NEURAL STEM/ PROGENITOR CELLS IN ADULT RATS AFTER FACIAL NERVE AVULSION

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Background: Neurogenesis occurs throughout life in two specific regions of the adult mammalian brain; the subventricular zone of the lateral ventricle and in the subgranular zone of the hippocampal dentate gyrus. After injury and pathological stimulations, adult neurogenesis has also been demonstrated in regions otherwise considered to be non-neurogenic.

Objectives: We investigated proliferation of nestin-immunoreactive (IR) neural stem/progenitor cells (NSPCs) in injured facial nuclei after facial nerve avulsion in adult rats.

Methods: The facial nerve of three-month-old Fischer 344 male rats was either avulsed or axotomized (1–4), and facial nuclei were histologically examined at different time points. Some animals were given 5-bromo-2'-deoxyuridine (BrdU) in their drinking water for four weeks after avulsion. Intact and injured facial nuclei from some animals were dissected, trypsinized and cultured in Neurobasal/B27 medium containing fibroblast growth factor-2 (FGF2) and epidermal growth factor (EGF).

Results: Two weeks after avulsion, motor neuron death became apparent (1–4) and nestin-IR cells proliferated in lesioned facial nuclei. BrdU-incorporated nestin-IR cells were observed in the facial nucleus on the avulsed side. Neither motor neuron death nor proliferation of nestin-IR cells was evident in facial nuclei after facial nerve axotomy. When intact facial nuclei were dissociated and cultured in the presence of FGF2 and EGF, neurospheres formed and nestin/NG2-IR NSPCs could be expanded and passaged for over eight months. *In vitro* proliferation of NSPCs derived from avulsed facial nuclei was much more vigorous than that from intact nuclei. These NSPCs differentiated to TuJ1, MAP2 and neurofilament-IR neurons, GFAP-IR astrocytes, and Rip, O4 and O1-IR oligodendrocytes in the presence of all *trans* retinoic acid and/or neurotrophic factors that include brain-derived neurotrophic factor and insulin-like growth factor-1.

Discussion and conclusions: We demonstrated that NSPCs proliferate following motor neuron degeneration caused by avulsion, but not axotomy, of the facial nerve in adult rats. These results indicate that the adult rat facial nerve avulsion model is a novel tool to investigate endogenous NSPCs in adult rat brain, and could be used to screen candidate therapeutics to promote *in vitro* and *in vivo* neurogenesis against motor neuron injury and motor neuron diseases in adult humans.

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P93 NEUROPROTECTIVE EFFECTS OF MULTI-VITAMIN THERAPY IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: There is no currently effective treatment for amyotrophic lateral sclerosis (ALS), although this disorder is a progressive neurodegenerative disease resulting in death within several years. Because recent evidence suggests that homocysteine (HC) is highly related to neurodegenerative disorders with ageing, we tried to elucidate the effects of multi-vitamin therapy on G93A SOD1 transgenic mice.

Objectives: The multi-vitamin therapy lowers homocysteine levels. We assessed whether multi-vitamin therapy reduced the risk of major symptoms in G93A SOD1 transgenic mice.

Methods: We treated this murine model (nine multi-vitamin treated mice, nine control mice) of ALS with multi-vitamin. The multi-vitamin therapy was composed of folic acid 1.97 mg/ml, pyridoxine 0.98 mg/ml and cyanocobalamin 0.1 mg/ml. We fed this complex with water to mice from 45 days of age. We performed the rotarod test and measured body weight from post-natal 10 weeks, weekly

Results: We found that multi-vitamin reinforcement significantly prolonged average lifespan and delayed disease onset with improvement of motor performance. The lifespan of mice with multi-vitamin was 138.3 $\pm$8.3 but control was 125.2 $\pm$9.7. We found that multi-vitamin treatment delayed disease onset and protected against loss of body weight. The onset of disease onset was 111.3 days for mice that took multi-vitamins but 96.6 days for controls. The body weight average of multi-vitamin treated mice was heavier than control mice. Especially, 16 weeks

of age had the largest difference between multi-vitamin mice (22.7 g) and control mice (20.8 g). However, body weight reduced from 16 weeks of age in multi-vitamin treated mice and 14 weeks of age in control mice. Finally, the result of the rotarod test showed that multi-vitamin treated mice tolerated it longer than control mice, from 12 weeks of age. At 13 weeks of age, multi-vitamin treated mice tolerated rotarod for 180 s but control mice tolerated it for 139.4 s. However, it did not significantly slow disease progression

Discussion and conclusions: These results suggest that multi-vitamin can be a potent therapeutic strategy for familial forms of ALS.

P94 L-CARNITINE SUPPRESSES THE ONSET OF NEUROMUSCULAR DEGENERATION AND INCREASES THE LIFESPAN OF MICE WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Mitochondrial dysfunction has been reported to participate in the pathogenesis of ALS. L-carnitine, an essential cofactor for the ß-oxidation of long-chain fatty acids, effectively inhibits various types of mitochondrial injury.

Objective: We examined the effect of L-carnitine on disease onset and survival of FALS-related $SOD1^{G93A}$ transgenic mice (FALS-Tg).

Method: L-carnitine was orally administered or subcutaneously injected to FALS-Tg. We analysed the effect of L-carnitine on neuromuscular injury, disease progression, and survival.

Results: We confirmed that the oral administration of L-carnitine prior to disease onset significantly delayed the onset of signs of disease (log-rank $p=0.0008$), delayed deterioration of motor activity, and extended life span (log-rank $p=0.0001$) in FALS-Tg. More importantly, subcutaneous injection of L-carnitine increased the life span of FALS-Tg (46% increase in males, 60% increase in females) even when given after the appearance of signs of disease.

Discussion and conclusions: L-carnitine may have beneficial, therapeutic effects on the improvement of neuromuscular functions in ALS.

P95 BENEFIT OF PYRUVATE IN G93A SOD1 MUTANT TRANSGENIC MICE

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that produces selective motor neuron death. Currently no effective pharmacological treatment is available for ALS.

Objectives: We investigated the neuroprotective effects of pyruvate in G93A SOD1 transgenic mice, the most widely used animal model of the familial form of ALS.

Methods:

Animal and sample preparation. Transgenic TgN (SOD1-G93A) 1GUR mice were obtained from the Jackson Laboratory (Bar Harbor, ME).

Survival and disease onset analysis. A resting tremor in G93A SOD1 transgenic mice is the initial symptom of disease onset. Subsequently, the mice developed gait impairment and paralysis of one (or both) hindlimbs.

Immunohistochemistry. Sections were incubated using the free-floating method (24 ~ 72 h, 4°C) with one of the following primary antibodies; GFAP, Bcl-2. Immunoreactivity was visualized using diaminobenzidine after signal amplification.

Motor Function Testing (Rotarod). Testing began by placing each mouse on a rod (diameter=4 cm) rotated at a constant speed of 16 rpm, and the time that a mouse stayed on the rod before falling off (3 min maximum) was recorded as a measure of motor function competence.

Results: Pyruvate delayed disease progression and prolonged the lifespan of G93A SOD1 transgenic mice, but did not delay disease onset. Pyruvate treated mice had better motor performance. Pyruvate treated and saline treated G93A SOD1 transgenic mice showed similar weight losses. Pyruvate treatment reduced GFAP and Nitrotyrosine immunoreactivities, and increased Bcl-2 immunoreactivity.

Discussion and conclusions: Our data demonstrate that pyruvate treatment has neuroprotective effects in G93A SOD1 transgenic mice. These neuroprotective effects may be associated with the inhibition of peroxynitrite-mediated nitration and apoptotic cell death.

P96 GSK-3β INHIBITOR SUPPRESSES SYMPTOM ONSET AND PROGRESSION IN ALS MOUSE MODEL

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Background and objectives: Glycogen synthase kinase (GSK)-3β has recently been emphasized as an important pathogenic mechanism in neurodegenerative diseases. Although the neuroprotective effects of GSK-3β inhibitors in Alzheimer's disease have been demonstrated, there has been no report of their effects on amyotrophic lateral sclerosis (ALS). This study was undertaken to evaluate the effects of a GSK-3β inhibitor on ALS model mice.

Methods: We treated each of 14 ALS mice with 0.5 ml normal saline mixed with 1.8 μg and 3.6 μg, respectively, of GSK-3β inhibitor per g of mouse and with only 0.5 ml normal saline intraperitoneally every week after 60 days of age (presymptomatic treatment).

Results: The GSK-3β inhibitor treatments significantly prolonged the time until symptom onset and lifespan, especially in the 1.8 μg/g dosage. The GSK-3β inhibitor inhibited the activity of GSK-3β in a concentration-dependent manner. Although this treatment preserved survival signal and attenuated death and inflammatory signals, the expression levels of intracellular signals were slightly different depending on the dose.

Conclusion: These data suggest that the inhibition of GSK-3β could be one potential therapeutic candidate for ALS, and that levels of GSK-3β inhibition might be very important in the therapeutic approach to ALS.

P97 INTRATHECAL INFUSION OF ANTI-HEPATOCYTE GROWTH FACTOR ANTIBODY EXACERBATES DISEASE PROGRESSION IN A RAT MODEL OF ALS

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Background and objectives: Hepatocyte growth factor (HGF) is one of the potent neurotrophic factors for motor neurons. A previous report had shown that overexpression of HGF in the nervous system could attenuate motor neuron loss and prolong the lifespan of transgenic mice with a mutant Cu/Zn superoxide dismutase (SOD1) gene. In addition, expression of HGF and its receptor, c-Met, was reported in surviving motor neurons in patients with amyotrophic lateral sclerosis (ALS). Thus, we have examined the possible significance of HGF as an endogenous disease-inhibitory factor using anti-HGF antibody in a rat model of ALS.

Methods: Expression of the endogenous rat HGF was examined in the lumbar spinal cords of Gly93Ala mutant SOD1 ($SOD1^{G93A}$) transgenic rats and their non-transgenic littermates at 10, 14, and 19 weeks old ($n=3$ in each group) with enzyme-linked immunosorbent assay (ELISA). Based on the results, rabbit polyclonal neutralizing antibody specific for rat endogenous HGF (5 μg/g (body weight)) was continuously infused into the subarachnoid space of $SOD1^{G93A}$ transgenic rats ($n=5$) for four weeks from the age at the beginning of endogenous HGF induction. As a control, normal rabbit IgG was administered instead of the antibody ($n=5$). Careful and daily observation revealed the onset of the disease and the survival endpoint. The endpoint used was the loss of righting reflex within 10 s. At the endpoint, we examined histopathology in the lumbar spinal cord by immunohistochemistry with neuronal and glial markers.

Results: In the $SOD1^{G93A}$ transgenic rats, the progressive induction of endogenous HGF occurred from the presymptomatic stage. At 14 and 19 weeks old, there was a 35% and 107% increase in the rat endogenous HGF levels compared with the age-matched non-transgenic littermates. Therefore, the anti-HGF antibody or normal rabbit IgG was administered to the $SOD1^{G93A}$ transgenic rats between the age of 14 and 18 weeks, continuously. In the anti-HGF-treated rats, the earlier onset ($p=0.18$), the faster progression ($p=0.03$), and the earlier survival endpoint ($p=0.046$) of the disease was observed compared with those of control rats. At the endpoint, the anti-HGF-treated rats showed the same pathology such as loss of ventral horn neurons, astrocytosis, and microgliosis as in the controls.

Discussion and conclusions: The present results showed that a blocking of endogenous HGF by intrathecal infusion of the specific antibody exacerbated the mutant SOD1-mediated disease. These results suggest that endogenous HGF may have an inhibitory role against ALS-like disease progression in this rat model and also in ALS patients. Therefore, the exogenous administration of HGF could be a therapeutic strategy for ALS in the future.

P98 DEVELOPMENT AND CHARACTERIZATION OF A MURINE INTRATHECAL CATHETERIZATION TECHNIQUE FOR DRUG DELIVERY TO THE CENTRAL NERVOUS SYSTEM

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Drug discovery and drug target validation in the field of amyotrophic lateral sclerosis research has been limited by the inability to consistently deliver drugs to the brain and spinal cords of animal models of the disease. Until recently

intracerebral ventricular (ICV) cannulation has been the only CNS drug delivery technique in use for mice, and preliminary evidence suggests that brain delivery of drugs does not ensure that they reach the spinal cord. The purpose of this study was to establish an intrathecal catheterization-infusion method for continuous delivery of drugs directly to murine spinal cords. We verified the catheter-pump localization and determined brain:spinal cord concentration ratios of drugs delivered by this method. Furthermore, we demonstrated that the surgical technique did not affect survival, neurological function, or chronically alter the profile of inflammatory mediators in spinal cord tissues of non-transgenic mice and SOD1 G93A transgenic mice.

P99 PROTEOMIC ANALYSIS OF NERVOUS SYSTEM OF SOD1-G93A MICE ADMINISTERED WITH POSSIBLE NEUROPROTECTIVE DRUGS

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Background: Several drugs are reported to prolong survival of SOD1-G93A mice. Proteomic analysis of tissue from drug-administered Tg mice might elucidate change of some key proteins.

Objectives: To identify some key proteins, which might play some roles in the neuroprotective effect of effective drugs.

Methods: Transgenic mice model for FALS, designated B6SJL-TgN(SOD1-G93A)1Gur purchased from Jackson Laboratory was used. Minomycin was administered daily by intra-peritoneal injection. Galanthamin-suspension was administered orally every day. Mice were sacrificed at three months old. Spinal cord tissues from minomycin or galanthamin-treated and control mice were thoroughly sonicated in 1 v/w of sample solution buffer consisting of 10 mM Tris HCl (pH 7.5), 9 M urea, 2% NP40, 2% mercaptoethanol, 1% protease inhibitor cocktail (Sigma) and centrifuged at 100,000 *g* for 1 h, and the supernatant was collected. The first dimension of gel electrophoresis was carried out using an immobilized pH gradient gel with a horizontal electrophoresis apparatus. The second dimension of gel electrophoresis was carried out on a 15% running gel in the presence of SDS. Protein spots on silver-stained 2-DE gels were digitized using a flatbed scanner. The image data were analysed using Melanie II software. Proteins with changed density in gels were identified with mass spectrometry.

Results: Several spots including GFAP, Vimentin, α internextin, Peroxiredoxin 5, HSP25, and 27 were elevated in mutant mice compared with non-transgenic mice, whereas NF-L, malate dehydrogenase, and Stathmin were decreased. Minomycin decreased HSP27 level and galanthamin also decreased HSP25 and 27 in challenged mice compare with sham operated mice.

Conclusion: The neuroprotective effect of minomycin and galanthamin might be mediated with heat shock molecules especially HSP25 and HSP27.

P100 CSF FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS DECREASES VOLTAGE ACTIVATED SODIUM CHANNEL NAV1.6 EXPRESSION IN DEVELOPING RAT VENTRAL HORN MOTOr NEURONS

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Amyotrophic lateral sclerosis (ALS) is a devastating human neurodegenerative disorder, which causes atrophy and weakness of skeletal muscles leading to death, the aetiology of which is still unknown. Our study has investigated the effects of CSF from ALS patients on voltage activated Nav1.6 sodium channel expression in developing rat ventral horn motor neurons. CSF obtained from ALS and non-ALS neurological patients was injected into three-day-old rat pups in the spinal subarachnoid space at the rate of 1 μl/2.5 min using a micro injector. The rats were sacrificed 48 h after CSF injection and spinal cord sections were processed for immunocytochemistry with Nav1.6 sodium channel antibody and also for cytochrome oxidase labelling. CSF samples from all six ALS patients significantly decreased Nav1.6 sodium channel expression compared to CSF from non-ALS patients. CSF from all six ALS patients also decreased cytochrome oxidase activity in the rat spinal motor neurons, which could be a sign of degeneration of motor neurons. Nav1.6 sodium channel produces rapidly activating and inactivating currents, which support the action potential generation. Decreased expression of this channel may suppress the electrical activity of the motor neurons, which may lead to degeneration of the neurons.

P101 AMPA AND CANNABINOID RECEPTOR TRAFFICKING IS ALTERED IN MOTOR NEURONS OF ALS MODEL MICE: IMPLICATIONS FOR EXCITOTOXICITY

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder involving the selective loss of spinal cord motor neurons. Excitotoxicity mediated by glutamate has been implicated as a cause of this progressive degeneration. In this study we used quantitative immumocytochemistry to comparatively measure the total cellular

expression and the plasma membrane localization of AMPA-type glutamate receptors (AMPARs) and cannabinoid receptors (CB1 and CB2) in spinal cord motor neurons. Wild-type and ALS model mice $SOD1^{G93A}$ were compared and specific subunit subtypes (GluR1 and GluR2) of AMPARs were followed. Motor neurons from $SOD1^{G93A}$ mice displayed variable expression patterns of glutamate AMPA receptor subunits during disease progression. Prior to disease onset, motor neurons from 40-day-old $SOD1^{G93A}$ mice showed increased GluR1 AMPAR surface expression compared to wild-type, but no change in total cellular expression when GluR1 was used to follow AMPAR localization. GlurR2 total cellular expression is found to be decreased at this time point. This finding is pertinent to excitotoxicity since the presence of properly edited GluR2 subunits in AMPARs blocks this channel from passing calcium while GluR2 lacking GluR1 homomers allows calcium passage. In a later stage of disease progression, in 120-day-old $SOD1^{G93A}$ mice, both plasma membrane and total cellular levels of GluR1 increased while total cellular GluR2 levels were decreased. The cannabinoid system in spinal cord has been shown to have protective effects on motor neuron survival, which may involve antioxidant and anti-excitotoxic functions. Here we measured CB1 and CB2 total cellular expression and plasma membrane localization alongside AMPAR expression at 40, 90, and 120 days. Total cellular level of CB1 was decreased while CB2 total cellular expression was increased at 120 days. Taken together, the changes of GluR1/GluR2 ratio in motor neurons in very young $SOD1^{G93A}$ mice suggest a possible mechanistic role for GluR2-lacking AMPA receptors in ALS disease initiation and progression. Observed CB1 and CB2 changes late in disease progression suggest an adaptive response to neurodegeneration induced by excitoxicity.

P102 ROLE OF SCHWANN CELL DYSFUNCTION IN MUTANT SOD1-MEDIATED NEURODEGENERATION

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Background: Mounting evidence from transgenic mice with cell restrictive, silenced or chimeric expression of mutant SOD1 suggests a potential non-neuronal contribution to motor neuron degeneration in FALS. Such studies of non-cell autonomous toxicity to date have focused on adjacent extraneuronal cells that occupy the spinal ventral horns. However, a recent evaluation of transgenic $SOD1^{G93A}$ mice revealed a neuropathological sequence consistent with a distal axonopathy (1), suggesting that peripheral cells such as skeletal myocytes or myelinating glia may influence disease. Schwann cells (SCs) are the major glial population of the PNS and interact intimately with lower motor neurons. Although principally concerned with myelination, SCs also modulate axonal transport, secrete neurotrophic factors and promote neuromuscular synapse regeneration following injury and disease. Thus, SC and motor neuron interactions may be relevant to FALS pathogenesis.

Objectives: To investigate the functional properties of SCs harbouring SOD1 mutations *in vitro* using primary transgenic $SOD1^{G93A}$ cultures or transfected cell lines and *in vivo* by generation and characterization of transgenic mice with SC targeted expression of mutant SOD1.

Methods: SCs were initially characterized in transgenic $SOD1^{WT}$, $SOD1^{G93A}$ and non-transgenic mice by immunohistochemical analysis of peripheral nerves for S100, myelin basic protein and p75 neurotrophin receptor ($p75^{NTR}$). Primary SCs were then isolated, purified and cultured from sciatic nerves of the neonatal mice above. In addition, the post-natal rat SC line (SCTM41) was stably transfected with EGFP-tagged wild-type and SOD1 mutant plasmids. Expression of neurotrophins, neuregulins and related receptors was analysed in both cell culture models using real time PCR, antibody arrays and immunoblotting. Conditioned medium and neuronal-glial co-culture experiments were also performed using stably transfected motor neuron-like (NSC-34) cells. Lastly, transgenic mice that overexpress $SOD1^{WT}$ or $SOD1^{G93A}$ exclusively in myelinating SCs were generated using a myelin protein zero promoter (P0-Cx32) splice construct and studied longitudinally for motor pathology and survival.

Results: Induction of $p75^{NTR}$ expression was observed in SCs from adult transgenic $SOD1^{G93A}$ mice, consistent with a denervated or pro-apoptotic phenotype accompanying axonal degeneration. Transgenic P0-Cx32 SOD1 mice were generated and these will be further characterized.

Conclusions: The characterization of these culture models and generation of transgenic mice with SC targeted expression of mutant SOD1 should clarify the role, if any, of peripheral myelinating glia in FALS aetiology and/or progression.

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P103 CONDITIONAL KNOCKOUT OF SOD2 IN POSTNATAL MOTOR NEURONS REVEALS IN-VIVO RESISTANCE TO MITOCHONDRIAL GENERATED SUPEROXIDE RADICALS

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Background: Oxidative stress has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), yet it

remains unclear whether oxidative stress is a major cause or merely a consequence of cellular dysfunction associated with ALS. Mitochondria are both a major source of reactive oxygen species (ROS) production as well as a major target of ROS-induced cellular injury. Although motor neurons in cell culture are shown to be vulnerable to cell death mediated via calcium influx after exposure to glutamate, it is unclear how motor neurons respond to the overproduction of mitochondrial-derived ROS *in vivo*.

Objectives: Mitochondrial localized superoxide dismutase (SOD2) is thought to play an important role in cellular defense against oxidative damage by ROS. Mice deficient in SOD2 die during embryonic or early postnatal development, which precludes analysis of a pathological role for superoxide in adult tissue. In order to circumvent the early lethality of SOD2 knockout mice, here we generated postnatal motor neuron-specific SOD2 knockouts, and analysed their pathophysiological alterations in motor neurons.

Methods: To generate postnatal motor neuron-specific SOD2 knockout mice, we crossed mice homozygous for floxed SOD2 alleles (1) with VAChT-Cre Slow mice in which Cre expression is restricted in postnatal somatomotor neurons (2). The spatial production of superoxide radicals was investigated by in situ detection of oxidized hydroethidine (HEt). Histological assessment was conducted by staining brain sections with cresyl violet or Fluoro-Jade B. The following marker antibodies were used to detect oxidative damage(s) in tissue sections: 8-OHdG, nitrotyrosine, malondialdehyde, SMI-31, and GFAP. To test whether SOD2-deficient motor neurons are more vulnerable to nerve injury, we employed unilateral transection of the hypoglossal nerve. Hypoglossal motor neuron survival was then assessed five weeks following transection. We also analysed the structural stability of the transected hypoglossal axons two days post-operation (Wallerian degeneration).

Results: SOD2 immunoreactivity was specifically lost in a subset of somatomotor neurons, resulting in enhanced superoxide production as revealed by massive oxidation and deposition of HEt in mitochondria. However, extensive histological examination revealed no signs of oxidative damage in animals up to 18 months after birth. Ultrastructural analysis of mitochondria in SOD2-deficient motor neurons revealed no degenerative changes such as swelling, disorganization of the cristae or vacuolar formation. Although motor neuron survival five weeks after hypoglossal nerve axotomy was unchanged, disorganization of distal nerve axons two days following injury was accelerated in SOD2-deficient motor neurons.

Discussion and conclusions: These data demonstrate that postnatal motor neurons are surprisingly resistant to oxidative damage from mitochondrial-derived superoxide radicals, but that such damage may sensitize axons to disorganization following nerve injury.

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P104 SOD1 AGGREGATES WITHIN MOTONEURONAL AXONS DO NOT ASSOCIATE WITH MITOCHONDRIA

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Background and objectives: Conversion of mutant SOD1 proteins into aggregates is thought to be responsible for the selective loss of motor neurons in SOD1-related ALS, although the mechanisms underlying aggregate-associated toxicity have not been clarified. Since SOD1 aggregates are detected in purified mitochondria and reported to be associated with an anti-apoptotic protein, Bcl-2, in symptomatic mutant SOD1 transgenic mice, dysfunction of mitochondria and/or an enhancement of mitochondria-dependent apoptosis is implicated in the pathogenic mechanism (1). In the course of study for the localization of SOD1 aggregates in $SOD1^{G93A}$ transgenic mice, we detected a significant amount of SOD1 aggregates within the axons in pre-symptomatic mice (2). Since a large portion of mitochondria is transported within motor neurons, we investigated whether SOD1 aggregates detected in axons were derived from transported mitochondria and associated with Bcl-2.

Methods: To isolate intact mitochondria or SOD1 aggregates derived from axons, not from cell bodies/dendrites of motor neurons, ventral white matter from the spinal cords of $SOD1^{G93A}$ and $SOD1^{wt}$ transgenic mice was dissected, homogenized, and subjected to Nycodenz-density gradient centrifugation. The conditions for homogenization differed in mitochondria- and SOD1 aggregation-isolation.

Results: To compare the distribution of mitochondria and SOD1 aggregates in Nycodenz fractions, we performed Western blotting using antibodies against SOD1 and several mitochondrial markers, and found that their distribution was clearly different. To study an association of SOD1 aggregates and Bcl2, the aggregate-enriched fraction was subjected to an immunoprecipitation with anti-SOD1 antibody followed by Western blotting with anti-Bcl2 antibody. In our system, Bcl-2 was not found to be co-immunoprecipitated by anti-SOD1 antibody.

Discussions and conclusions: Since the distribution of mitochondria and SOD1 aggregates was different in Nycodenz-density gradient fractionation, a majority of mitochondria transported within axons is unlikely to involve the SOD1 aggregates. This result was supported by the immunoprecipitation analysis, in which association of SOD1 and Bcl-2 was not detected in the SOD1 aggregate-enriched Nycodenz fraction. We alternatively detected certain axonal transport-related proteins which were co-immunoprecipitated by anti-SOD1 antibody. From these and other data, we concluded that the SOD1 aggregates within axons are not co-transported with mitochondria, but transported by unknown systems.

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P105 ALTERED UBIQUITINATED PROTEIN EXPRESSION IN WOBBLER AND TRANSGENIC SOD1^{G93A} SPINAL CORD: A PROTEOMIC ANALYSIS

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Background: Ubiquitinated inclusions (UbIs) occur in neurons of patients with ALS, and of certain models of ALS, including wobbler (wr) and transgenic SOD1^{G93A} (TgSOD1^{G93A}) mice. This probably reflects abortive proteolysis of one or more abnormal proteins, although the contents of the inclusions are not known. Although potentially protective by sequestering toxic proteins, the UbIs may inadvertently trap normal proteins which prevent their functioning and results in further cellular injury.

Objectives: To identify by mass spectrometry based proteomics ubiquitinated proteins in the spinal cord of wr and TgSOD1^{G93A} mice expressing a 6 x His-tagged ubiquitin transgene. The 6 x His tag allows enrichment of ubiquitinated proteins, either within inclusions or not, for subsequent characterization. This may provide new insights into disease pathogenesis and novel therapeutic targets.

Methods: Cervical and lumbar spinal cord was obtained from 56 day-old mutant wr and wild-type littermates, as well as 90 day-old TgSOD1^{G93A} mutant and nonTgSOD1^{G93A} control mice. 6 x His-tagged ubiquitinated proteins were enriched from these regions by nickel-coated ProteinChips® and analysed in duplicate by surface enhanced laser desorption/ionization mass spectrometry (SELDI-TOF-MS). Evaluation of the spectra was performed using the Ciphergen software (Ciphergen Biosystems, Inc.). Comparisons were made between mutant and control mice of each group (wr vs. wild-type and TgSOD1^{G93A} vs. nonTgSOD1^{G93A}) and also between groups (wr vs. TgSOD1^{G93A}).

Results: Significant alterations were detected in 17 protein mass peaks of wr vs. wild-type spinal cord. We also identified increased levels of peptide fragments in the wr sample corresponding to the molecular mass of the Vps54 protein, known to be mutated in wr mice. Nine protein mass peaks were significantly altered in TgSOD1^{G93A} mice compared to their non-TgSOD1^{G93A} controls, including increased SOD1, which was not altered in the wr sample. Except for two mass peaks that were increased (6.78 and 7.93 kDa) and one that was decreased (12.2 kDa), in both wr and TgSOD1^{G93A} mice all other peak identities were unique to each group. In addition, two protein peaks exhibiting increased levels in TgSOD1^{G93A} mice were decreased in mutant wr mice.

Discussion and conclusions: Our proteomic data reveal alterations of specific ubiquitinated proteins in the spinal cord of the wr and TgSOD1^{G93A} mouse models of ALS. While some of the proteins involved were similar between the wr and mutant SOD1 groups, most were specific to the model, and sometimes altered in opposite directions. This suggests more uniqueness than commonality at the protein level in the pathogenesis of motor neuron degeneration in these two mouse models. We are determining the protein identity of each protein and applying SELDI-TOF-MS methods to identify specific contents of isolated ubiquitinated inclusions. Our study indicates that mass spectrometry based proteomics can identify protein alterations and help elucidate disease mechanisms in mouse models of ALS.

P106 INTRANEURONAL ACCUMULATION OF UBIQUITINATED INCLUSIONS IS ASSOCIATED WITH IMPAIRED PROTEASOME FUNCTION IN WOBBLER MOTOR NEURON DEGENERATION

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Background: Ubiquitinated inclusions (UbIs) occur in neurons of patients with ALS, implicating ineffective proteolysis by the ubiquitin-proteasome system (UPS). Such inclusions, which may be neurotoxic, can contain an aberrant protein that would be harmful if not sequestered from the cell. The recent discovery that a mutation in *Vps54,* which is involved in endosomal trafficking, causes neuronal degeneration in the wobbler (wr) mouse is of interest because we find UbIs in neurons of cortex (Cx) and cervical spinal cord (CSC). However, how this mutation results in neuronal degeneration and formation of these inclusions is unknown.

Objectives: To characterize intraneuronal UbIs and to assess proteasome activity in wr CSC and Cx, in order to determine whether the UPS is dysfunctional.

Methods: CSC and Cx of BL6NZB wr mice and their wild-type littermates were examined at presymptomatic (14 days) and early symptomatic (28 days) stages by immunocytochemical and biochemical methods to characterize the UbIs, their associated proteins, and relationship to organelles. We also assessed chymotrypsin-, trypsin-, and caspase-like enzymatic activities of the 20S proteasome.

Results: UbIs visible by light microscopy develop in the CSC and Cx of the wr mouse, but not its wild-type littermate, between 16 and 18 days of age. However, ubiquitinated proteins identified by size exclusion filter trapping already appear at 14 days. Although closely

associated with endoplasmic reticulum, Golgi apparatus, and lysosomes, UbIs are not colocalized with them. Proteasome 20S activity is significantly reduced ($p<0.01$) in wr relative to wild-type, primarily for chymotrypsin-like activity at 28 days (CSC, –18%; Cx, –25%), but also at 14 days (CSC, –19%; Cx, –16%). Lesser, although still significant, reductions occur in the other enzymatic activities at 28 days.

Discussion and conclusions: Accumulation of ubiquitinated proteins precedes disease onset in wr CSC and Cx in a pre-aggregate state before forming visible inclusions. Although formed inclusions may protect the cell by sequestering harmful proteins, the pre-aggregate may be detrimental, and inhibit proteasome activity. We found predominantly chymotrypsin-like but also trypsin- and caspase-like enzyme activities to be significantly reduced in CSC and Cx as early as 14 days, when mice appear normal. This indicates that UPS dysfunction precedes disease onset and probably contributes to accumulation of UbIs and neurodegeneration in the wr mouse.

P107 REDUCTION OR ABSENCE OF NORMAL PRION PROTEIN ACCELERATES DISEASE IN G93A SOD-1 MICE

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Background: Misfolded prion proteins have been implicated in several neurodegenerative diseases of animals and man. However, the normal function of the prion protein has remained more elusive. PrP null mice breed and develop normally, and seemingly demonstrate few abnormalities in adult life. Recently the prion protein has been shown to be neuroprotective in hypoxic/ischaemic injury and epileptogenesis, with anti-apoptotic and anti-excitotoxic activity. Since these pathological processes have been implicated in ALS, PrPc may have beneficial action in ALS. However, there is also a possibility that PrP^{c} could play an unexpected detrimental role in ALS, as PrP^{c} overexpression can lead to caspase 3 activation and cell death independent of PrP^{sc} formation, in a process accelerated by proteasomal inhibition (which might occur in ALS).

Objectives: We wished to test whether transgenic mice overexpressing the human G93A SOD1 transgene but lacking one or both copies of the PrP gene demonstrated improved or worsened survival compared to those expressing normal PrP.

Methods: PrP null mice were obtained as a gift from Dr. Jean Manson of the Institute for Animal Heath in Edinburgh. These mice were crossed with heterozygote G93A SOD1 mice and the F1 generation bearing the SOD1 transgene were backcrossed against PrP null mice. In this way three groups of G93A SOD1 mice can be produced: mice homozygous for the deleted *PrP* gene, mice heterozygous for the deleted *PrP* gene, and wild-type PrP. We compared disease onset and survival in the three groups.

Results: Very few SOD1 mice homozygous for the PrP deletion could be bred, and it is likely that there is decreased survival in utero. Mice heterozygous for the PrP deletion ($n=16$) had significantly accelerated disease onset compared to wild-type ($n=8$) (103 days vs. 112 days, respectively), and a major reduction in survival (113 days vs. 144 days, $p<0.001$).

Conclusions: The normal cellular prion protein plays a protective role in the SOD-1 mouse model of ALS. We are presently looking for loss-of-function mutations or deletions in the prion protein in patients with ALS. It is unclear whether interventions aimed at enhancing PrPc levels would be beneficial.

P108 DYSREGULATION OF LIPIDAEMIA IN ANIMAL MODELS AND ALS PATIENTS

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Background: Our work has previously shown that skeletal muscle hypermetabolism is a hallmark of the pathology developped by mutant SOD1 mice, an animal model of amyotrophic lateral sclerosis (ALS).

Objectives: We analyzsed lipid metabolism in mutant SOD1 mice and in a cohort of 369 patients suffering from ALS.

Methods: We used biochemical and physiological analysis of lipid metabolism coupled with gene expression measurements using real time RT-PCR.

Results: We show here that SOD1(G86R) mice display decreased blood lipids. Hypolipidaemia was not due to intestinal or hepatic dysfunction but rather to increased triglycerides-rich lipoproteins clearance by peripheral tissues including skeletal muscle. Moreover, mutant SOD1 mice muscles displayed neutral lipid accumulation, increased expression of genes involved in lipoprotein clearance as well as muscle aggregates of mutant SOD1. Contrary to the animal model, ALS patients were hyperlipidaemic but, similar to the situation in mice, a better survival of ALS patients was associated with an abnormally high LDL/HDL ratio.

Conclusions: Altogether, our results show that alterations in lipoprotein metabolism are associated with amyotrophic lateral sclerosis and contribute to neurodegeneration.

P109 NUCLEOCYTOPLASMIC TRANSPORT INVOLVEMENT IN THE SPINAL CORD OF A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Nucleocytoplasmic transport is essential for maintaining cell viability and cellular functions. Transport of proteins and nucleic acids between the nucleus and the cytoplasm occurs through nuclear pore complexes (NPCs). NPCs have been only recently investigated in patients with autoimmune, neoplastic, viral or hereditary disorders. Regarding neurodegenerative diseases, very recently Sheffield et al. reported the first evidence for the involvement of NPCs in Alzheimer's disease.

Objectives: To examine whether dysfunction of the nucleocytoplasmic transport system is involved in the pathomechanisms of ALS.

Methods: Lumbar spinal cord sections from G93A SOD1 transgenic female mice at 8–20 weeks of age, and from age-matched female wild-type littermates were investigated immunohistochemically. The following primary antibodies were used: monoclonal antibodies against nucleoporin p62 (Nup62), against karyopherin alpha2/Rch-1 (importin alpha), against histone H1, and against caspase-3, and polyclonal antibodies against the importin beta family (karyopherin beta1 (importin beta), karyopherin beta2 (transportin), karyopherin beta3 (importin 5)), and against beta-catenin.

Results: The antibody against Nup62, one of the structural proteins of NPC, labeled the nuclear rim because of regular contours of most of the lumbar anterior horn cells in the control mice. By contrast, this antibody demonstrated the nuclear irregularity with disrupted contours of the surviving anterior horn cells of the transgenic mice, irrespective of their age. Using antibodies against the importin beta family, the major carrier proteins of nucleocytoplasmic transport, and those against their adapter protein, importin alpha, the immunoreactivities were decreased within the nuclei and increased within the cytoplasm of a subset of the remaining anterior horn cells of the transgenic mice. In addition, Lewy body-like hyaline inclusions were invariably identified with these antibodies. Furthermore, the immunoreactivities for histone H1 and beta-catenin, the representative cargo proteins transported by importin beta family-dependent and family-independent nucleocytoplasmic transport pathways, respectively, were demonstrated similarly to those for importin beta family and importin alpha. The altered distributions of these proteins were not associated with caspase-3 expression. Chronological quantitative analysis of sections from the transgenic mice revealed a progressive decrease in the proportion of the anterior horn cells exhibiting a more intense reactivity for these carrier and cargo proteins in the nucleus than in the cytoplasm. In contrast, we found that anterior horn cells with the immunoreactivity in their cytoplasm being more pronounced than that in their nucleus were obviously increased in number along with the disease progression.

Conclusions: Our present results imply that dysfunctional nucleocytoplasmic transport could be involved in the pathomechanisms underlying ALS.

P110 ANALYSIS OF THE ROLE OF DYNEIN MUTATIONS IN ATTENUATING THE PHENOTYPE OF MUTANT-SOD1 TRANSGENIC MICE

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Background: Cu/Zn $SOD1^{G93A}$ transgenic mice develop an ALS-like phenotype, which is characterized by motor neuron degeneration and muscle paralysis. Previously, we demonstrated that a missense point mutation in the gene encoding the heavy chain subunit of cytoplasmic dynein causes degeneration of motor neurons in the legs at odd angles (*Loa*) mouse (1). We also showed that double mutant (*Loa*/$SOD1^{G93A}$) transgenic mice have a significant improvement in $SOD1^{G93A}$-mediated disease accompanied by a reduction in motor neuron degeneration (2).

It is possible that the improvement in the phenotype of the double mutants is due to interaction of $SOD1^{G93A}$ with the dynein complex.

Objectives: 1) To analyse the dynein complex in wild-type and *Loa* mice and examine whether mutant SOD1 interacts with the dynein complex. 2) To elucidate at the molecular level the role of the *Loa* mutation in attenuating the phenotype of $SOD1^{G93A}$ transgenic mice.

Methods: We have been using protein-protein interaction assays, such as immunoprecipitation and pull-down assays followed by Western blotting and mass spectrometry to dissect the dynein complex in *Loa* mice and test the possibility of its interaction with $SOD1^{G93A}$ *in vivo* and *in vitro*.

Results: Our preliminary pull-down assays using bacterially expressed wild-type and several mutant SOD1 proteins show that there is a potential interaction between mutant SOD1 and the dynein complex from wild-type and *Loa* brain homogenates with a possible higher affinity towards mutant dynein. In addition, using an anti-dynein antibody and brain tissue extracts from double mutant (*Loa*/$SOD1^{G93A}$) transgenic mice, we have co-immunoprecipitated $SOD1^{G93A}$ protein with the dynein complex. Nano-spray liquid chromatography/mass spectrometry (LC/MS) of the dynein complex from brains of wild-type and *Loa* mice immunopreciptitated by anti-dynein

antibodies have identified some interesting interacting proteins including GAPDH, whose importance in neuronal apoptosis and neurodegenerative disorders is emerging.

Discussion: Our work is in progress towards verifying these findings.

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P111 ROLE OF ASK1 IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS-LINKED MUTANT SOD1 TOXICITY

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Background: Some familial ALS (FALS) cases are associated with dominantly inherited missense mutations in the Cu/Zn-superoxide dismutase-1 (SOD1) gene. A recent *in vitro* study suggested that the SOD1 mutant-induced death is mediated by the Fas receptor and apoptosis signal-regulating kinase 1 (ASK1) (1).

Objectives: To characterize the role of ASK1 in the pathogenesis of FALS, we crossed ASK1 knockout mice with G93A-SOD1 transgenic (Tg) mice (G93A Tg/ $ASK1^{-/-}$).

Methods: G93A-SOD1 Tg mice were crossed with ASK1 knockout mice (2). Motor performance of the mice was evaluated in the rotarod test as previously described (3). NSC34 motoneuronal cells were transfected with pEF-BOS vector, wild-type-SOD1, or G93A-SOD1 cDNA and phosphorylation states of c-Jun N-terminal kinase (JNK) and p38 MAPK were detected with immunoblot analysis.

Results: There was no difference in the disease onset between the control mice (G93A/$ASK1^{+/+}$ mice) and G93A/$ASK1^{-/-}$ mice. Mean survival of G93A/$ASK1^{-/-}$ mice, however, was significantly longer than that of the control mice. Transfection with the G93A-SOD1 gene led to earlier and continuous JNK activation while it did not lead to significant activation of p38 MAPK in NSC34 cells.

Discussion and conclusions: Enetic disruption of ASK1 from ALS mice improved motor performance and prolonged survival without delaying disease onset, suggesting that ASK1 is involved in the disease progression of FALS. Mutant SOD1 induced the JNK activation rather than the activation of p38 MAPK in NSC34 cells. Thus, the Fas/ASK1/JNK pathway might be involved in motoneuronal death in FALS. Therefore, molecules constituting the signaling pathway would be new targets of a remedy for ALS.

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P112 COMPARATIVE STUDIES OF MOTOR NEURON DISEASE IN MICE TRANSGENIC FOR HUMAN WTSOD1 AND MUTATED SOD1

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Background: Superoxide dismutase 1 (SOD1) is associated with familial amyotrophic lateral sclerosis (ALS) and may well play an important role in sporadic ALS.

Objective: Mice transgenic for mutated D90A, G127X, G93A, G93A del, G85R and wild -type human SOD1 as well as non-transgenic control mice C57BL/BJBom were compared to reveal common traits and differences in development and progress of motor neuron disease.

Methods: Mice were sacrificed at preselected intervals and at terminal stage. Three levels of the spinal cord as well as the brainstem, cerebellum and brain were studied. Genotyping and SOD activity analysis were performed. Histopathology and immunohistochemistry using polypeptide antibodies directed against exon 1 and 5 of human SOD1 were performed.

Results: Blood levels and activity of SOD1 seem to be unrelated to disease progression and lifespan. In all transgenic mice, small SOD1 positive inclusions were found in the ventral horns from young age, and accumulated throughout life. SOD1 positive aggregates also accumulated in both glial cells and in white matter of the spinal cord. Vacuolization, gliosis and hyperchromatic cells were also found, but the extent varied between strains. In terminal mice SOD1 is also present in axons and in peripheral nerves.

SOD1 positive inclusions are also seen in the motor areas of the brain and brainstem. Large aggregates were found in the cerebellum and in white matter of the brainstem. Hyperchromasia, vacuolization and gliosis were found in parts of the brain, the extent varied between the different mouse strains.

Discussion: Aggregation of SOD1 in the central nervous tissue appears to be related to severity of disease. Degenerative features such as vacuolization and gliosis precede phenotypic alterations. Changes are seen not only in motor areas but also in higher centres of the

telencephalon. Our studies indicate that the SOD1 gain-of-function is due to aggregation.

P113 HUMAN SOD1 LACKING NATIVE AS WELL AS CARRYING NON-NATIVE DISULPHIDE BONDS ENRICHED IN SPINAL CORD OF MURINE ALS MODELS

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Background: Over 100 mutant SOD1s associated with ALS cause disease by an unknown cytotoxic mechanism. SOD1 is present in higher levels in most peripheral organs than in motor areas of the CNS. The murine transgenic ALS models express variable amounts of human SOD1 in spinal cords, from one-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 the spinal cord are disulphide-reduced, inactive, probably misfolded 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). One hundred per cent of the G127X and 80% of G85R in the spinal cords binds to HIC columns. In the more stable SOD1 models, G93A and D90A, subfractions of the SOD1 in spinal cord extracts are bound to the HIC columns, 5% and 1%, respectively. Much less of the SOD1 in brain and peripheral organs is bound in the HIC. In the G93A mice there was no change in the binding proportion from 20 days until they were moribund at 124 days. The hydrophobic soluble SOD1, long-term enriched in spinal cords, could be responsible for the cytotoxicity.

Objectives: The disulphide status of SOD1 is of great importance for the folding of the protein. Reduction of the intrasubunit disulphide bond results in a destabilization of SOD1 that could lead to HIC affinity. This study attempts to reveal the disulphide status of the hydrophobic subfractions of SOD1.

Methods: Brain, spinal cord, liver and kidney homogenates from transgenic mice were centrifuged at 20,000 *g* for 20 min at 4°C, the supernatants were collected and subjected to octyl-sepharose HIC. Multiple SOD1 variants expressed in E. coli with different cysteins mutated, were denatured with gunidinium chloride, reduced with TCEP and metal-deprived with DTPA. They were then boiled in sample buffer and allowed to oxidize and disulphide shuffle. Reduced and non-reduced Western immunoblots were used to analyse eluates for human SOD1.

Results: All recombinant SOD1s, tissue homogenates and HIC binding fractions show a single band of the expected molecular weight on reduced SDS gels. On non-reduced gels, tissue homogenates contain both disulphide reduced and oxidized protein as seen before. The recombinant proteins show that SOD1 can disulphide shuffle to form non-native disulphide bonds with all four cysteins involved. HIC binding SOD1 includes both disulphide-reduced SOD1 and species with non-native disulphide bonds.

Discussion: Disulphide shuffling in the reduced cytosol is surprising and might hint that HIC binding SOD1 can be of other cellular origin than cytosolic. These hydrophobic subfractions might be cytotoxic directly via reaction with critical components of the cell, or via aggregation.

P114 EVIDENCE FOR ZINC-DEFICIENT SOD BEING THE TOXIC FORM RESPONSIBLE FOR ALS

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We have shown that the ALS-associated mutant SODs have a decreased affinity for zinc, making them more prone to becoming zinc-deficient. Furthermore, the copper in zinc-deficient Cu,(–) SOD catalyses the nitric oxide-dependent formation of peroxynitrite and nitration of tyrosine, which activates apoptosis in motor neurons. Using a novel mass spectrometric assay, we have directly measured zinc-deficient SOD from the disease affected spinal cords of transgenic rats. Zinc-deficient SOD was about one-third of the Cu/Zn SOD present and four times greater than the endogenous rat SOD. Zinc-deficient SOD was not present in the non-disease affected brains of the same animals nor in wild-type SOD overexpressing rats. In addition, we have solved the X-ray structure of zinc-deficient SOD, which provides insights into the increased redox activity of the active site copper as well as to why zinc-deficient SOD is more prone to aggregation. Inhibition of aggregation by forming heterodimers with Cu/Zn SOD increases the toxicity of zinc-deficient SOD, which may explain in part the dominant action of ALS mutants. Paradoxically, growing evidence indicates that aggregation of SOD may be a protective mechanism to remove zinc-deficient SOD. In summary, the loss of zinc from either wild-type SOD or ALS mutant SOD offers a reasonable explanation for how this key antioxidant enzyme can be involved in sporadic and familial ALS.

P115 THE TOXIC EFFECT OF MUTANT SOD1 ON MOTOR NEURON OUTGROWTH IN THE ZEBRAFISH

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Background: ALS is a devastating neurodegenerative disease that leads to paralysis and death due to progressive loss of upper and lower motor neurons. The mechanism leading to this degeneration is not understood. While most of the cases of ALS are sporadic (SALS), almost 10% are familial (FALS). Approximately 20% of familial ALS is linked to mutations in the Cu/Zn superoxide dismutase (SOD1), of which over 60 different mutations are described. It is assumed that these mutations cause a toxic gain of function rather than a decreased dismutase activity.

Several animal and cell culture models have focused on the pathogenic role of mutant SOD1 in FALS. In spite of these strenuous efforts, the mechanisms underlying its effects remain obscure.

Objectives: This study explores the toxicity of mutant SOD1 on zebrafish motor neurons. In zebrafish it is known that motor neurons originate in two different waves. First, three primary motor neurons per spinal cord hemisegment appear. These neurons send out their axons which follow a distinct pathway. A few hours later, 30 secondary motor neurons per spinal cord hemisegment are generated. These will produce axons that will follow the pathway paved by the primary motor neurons.

Methods: Transient overexpression of wild-type and different mutant human SOD1 was induced by the microinjection of mRNA. The effect on motor neuron biology was evaluated by determining defects in primary motor neurons, in particular branching and measuring axonal outgrowth of 30-h-old embryos.

Results: Embryos injected with three different SOD1 mutant (G93A, G37R and A4V) mRNAs showed more branched and shorter axons compared to their $SOD1^{WT}$mRNA injected littermates. Furthermore, using different concentrations of RNA, we proved this effect on axonal outgrowth to be dose dependent.

Discussion and conclusions: We conclude that mutant SOD1 is toxic for embryonic zebrafish motor neurons. This new model for acute SOD1 neurotoxicity creates a broad range of opportunities. The effect of mutant SOD1 on zebrafish movement behaviour will be evaluated. Injecting mutant SOD1 mRNA in the many mutants obtained from large mutagenesis screens will facilitate the detection of modifying genes. In the future we are also planning to use this model to perform a small compound screening for the testing of a wide variety of compounds on mutant SOD1 neurotoxicity.

The zebrafish experimental framework integrates the strengths of different existing animal and cell culture models and has the potential to generate new insights in ALS pathogenesis and treatment.

P116 KNOCKING DOWN THE *ALS2* GENE IN ZEBRAFISH LEADS TO DEVELOPMENTAL ABNORMALITIES AND SWIMMING DEFICITS

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Background: The *ALS2* gene encodes a protein, alsin, which contains multiple motifs with homology to the guanine-nucleotide exchange factor. To date, there are ten reported mutations in the *ALS2* gene, which all lead to either 1) amyotrophic lateral sclerosis (ALS), where both upper and lower motor neurons are affected, or 2) primary lateral sclerosis (PLS), or 3) infantile onset ascending hereditary spastic paraplegia (IAHSP), where only upper motor neurons are affected. Furthermore, the molecular features and function of alsin have not been experimentally determined and the pathological mechanisms by which a loss of alsin function leads to a selective dysfunction and degeneration of motor neurons in three distinct motor neuron diseases remain unknown.

Objective: To generate a zebrafish model of ALS2 in order to address the function and molecular features of alsin, as well as its involvement in neuronal dysfunction.

Methods: The ALS2 zebrafish ortholog was identified using the Ensembl's gene homology prediction program (http://www.ensembl.org, build Zv3). In an attempt to address the function of alsin in zebrafish, we performed a loss-of-function study using antisense morpholino oligonucleotide (AMO)-mediated gene knockdown. AMO sequences were designed to be complimentary to the region of translational initiation of the zebrafish ALS2 ortholog, in order to inhibit protein translation and block endogenous alsin production. The appropriate AMOs were injected into one- to two-cell zebrafish embryos and the resulting larvae were monitored for the presence/absence of a response to touch phenotype.

Results: Zebrafish larvae resulting from the injection of antisense morpholino oligos directed against the start codon of the z*ALS2* gene presented with an obvious developmental and behavioural phenotype, including swimming impairment after proper touch response and background adaptation. This phenotypic observation was absent in control animals and thus seems to be a direct result of the knocking down of zebrafish alsin expression.

Discussion: According to the preliminary results presented here, the ALS2 knocked-down zebrafish would be an excellent animal model to study the pathophysiological

mechanisms associated with the neuronal dysfunction observed in ALS2.

P117 NAD+ AND WALLERIAN DEGENERATION REVISITED: DISSECTING THE FUNCTIONAL DOMAINS OF THE WLDS PROTEIN

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Background: The slow Wallerian degeneration protein, Wld^S, is a potent and unique neuroprotective factor for axons and synapses. It delays axon degeneration after injury by 10-fold, and in *pmn* mice it delays onset of symptoms and increases lifespan by 40%. It also protects neuromuscular junctions in young G93ASOD1 transgenic mice, a widely used model of familial ALS. Its potential effect in sporadic human ALS remains unknown.

Objectives: Understanding the Wld^S neuroprotective mechanism may reveal novel targets for therapy. Wld^S is a fusion protein containing 70 N-terminal amino acids (N70) of multiubiquitination factor Ube4b and full-length nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1). It was recently proposed that Nmnat1 activity is solely responsible for axon protection, but the relevance of these data *in vivo* was not tested. Other studies highlight the importance of the ubiquitin proteasome system (UPS) in regulating Wallerian degeneration, so we tested whether the N-terminal sequence of Wld^S is truly dispensable *in vivo*.

Methods: Using the same approach that previously led us to identify the *Wld*^{S} gene, we generated and characterized five lines of Nmnat1 overexpressing transgenic mice. We confirmed that Nmnat1 was overexpressed in the nuclei of lumbar spinal cord motor neurons and in dorsal root ganglia, both of whose axons project to the sciatic nerve, and that total Nmnat activity closely matched that of *Wld*^{S} mice. We then cut sciatic nerves to assess Wallerian degeneration by electron microscopy and other methods. We also pulled down a binding partner for N70 from mouse brain, and confirmed its physiological relevance by immunoprecipitation and colocalization studies.

Results: All five Nmnat1 overexpressing transgenic lines failed to reproduce the Wld^S phenotype. In contrast, even a low dose of Wld^S consistently slowed Wallerian degeneration in transected sciatic nerves. In our hands, transgenically overexpressed Nmnat1, exogenous NAD^+ and resveratrol were similarly ineffective *in vitro*. Interestingly, the N70 domain required for neuroprotection directly binds to valosin containing protein (VCP; p97/Cdc48), an AAA-ATPase with diverse cellular roles including a pivotal role in the UPS. Transgenic mice have been generated to test whether VCP mediates the Wld^S mechanism.

Conclusion: Nmnat1 does not protect injured axons *in vivo* when expressed at similar levels to Wld^S, and we find its protective effect *in vitro*, if any, to be far weaker than that of Wld^S. More N-terminal sequence in Wld^S is therefore required for the protective phenotype. VCP, a binding partner for N70, is a good candidate to mediate this phenotype, given its critical role in the UPS. As axons and synapses degenerate early in ALS, it is essential to complete our understanding of how they degenerate and how agents such as Wld^S and its downstream mediators might intervene.

P118 PHYSIOLOGICAL ANALYSIS OF THE PHENOTYPE OF A YAC TRANSGENIC MOUSE MODEL OF SPINAL BULBAR MUSCULAR ATROPHY (SBMA)

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Background and objectives: Spinal and bulbar muscular atrophy (SBMA) is a progressive human motor neuron neurodegenerative disorder due to a polyglutamine expansion in the androgen receptor (*AR*) gene. An SBMA YAC transgenic mouse model (AR100) has previously been generated expressing the full length human *AR* gene containing 100 CAG repeats, leading to a neuromuscular phenotype (1). Here we characterize the phenotype of both homozygous and heterozygous AR100 transgenic mice by *in vivo* physiological analysis of muscle function and motor unit survival at a late symptomatic stage of disease.

Methods: At 13 and 17 months, respectively, homozygote and heterozygote mice became clearly symptomatic showing marked locomotor deficits and were prepared for *in vivo* analysis of hindlimb muscle function. The mice were anaesthetized and the distal tendons of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles were attached to isometric force transducers and motor unit survival and maximum muscle force elicited by direct stimulation of the sciatic nerve was assessed in both hindlimbs. The fatiguability of EDL was assessed by recording the response to repetitive nerve stimulation. Muscles were then removed for analysis of muscle phenotype and innervation and the spinal cord processed for analysis of motor neuron survival.

Results: Compared to wild-type control mice, TA muscles were approximately 50% weaker and EDL approximately 40% weaker in homozygote and heterozygote AR100 mice at 13 and 17 months, respectively ($p < 0.01$). There was no significant difference in muscle force between homozygote and heterozygote mice, indicating that the disease process is accelerated in homozygote mice. There was also a significant reduction in the number of motor units innervating EDL so that 25 and 22

motor units survived in homozygotes and heterozygotes, respectively, compared to 30 in wild-type mice ($p < 0.05$). Moreover, the EDL muscle phenotype was altered so that this normally fast fatiguable muscle became fatigue-resistant in AR100 homozygote and heterozygote mice – a change in muscle phenotype associated with motor neuron degeneration. Histological analysis reveals marked muscle denervation.

Conclusions: Our results indicate that both homozygote and heterozygote AR100 mice develop significant muscle weakness, a loss of motor units accompanied by a change in muscle phenotype, reflecting the observed neuromuscular phenotype previously described (1). These changes are more severe and of earlier onset in the homozygote mice. Our results provide strong physiological evidence that these mice are a representative model of human SBMA.

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THEME 7 *IN VITRO* EXPERIMENTAL MODELS

P119 FOLDING OF CU/ZN SOD REVEALS STRUCTURAL HOTSPOTS FOR GAIN OF NEUROTOXIC FUNCTION IN ALS: PARALLELS TO PRECURSORS IN AMYLOID DISEASE

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease linked to misfolding of the ubiquitous enzyme Cu/Zn superoxide dismutase (SOD). In contrast to other protein-misfolding disorders with similar neuropathogenesis, ALS is not always associated with the *in vivo* deposition of protein aggregates. Thus, under the assumption that all protein-misfolding disorders share at primary level a similar disease mechanism, ALS constitutes an interesting disease model for identifying the as yet mysterious precursor states from which the cytotoxic pathway emerges. In this study, we have mapped out the conformational repertoire of the apoSOD monomer through analysis of its folding behaviour. The results allow us to target the regions of the SOD structure that are most susceptible to unfold locally under physiological conditions, leading to the exposure of structurally promiscuous interfaces that are normally hidden in the protein's interior. The structure of this putative ALS precursor is strikingly similar to those implicated in amyloid disease (1).

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P120 THE ROLE OF CYSTEINE 111 RESIDUE IN MUTANT COPPER/ZINC SUPEROXIDE DISMUTASE (SOD1)

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Background: Mutations in the gene encoding human copper/zinc superoxide dismutase (SOD1) cause familial amyotrophic lateral sclerosis (FALS). It has been proposed that neuronal cell death might be brought about by oxidative stress through the inappropriate redox activity of Cu associated with mutant SOD1. On the other hand, genetic ablation of copper chaperone for SOD1 (CCS), which delivers copper to the active site of SOD1, cannot rescue the disease. In previous studies, we proved mutant SOD1 possessed higher affinity for Cu than wild-type by immobilized copper affinity chromatography (Cu-IMAC) probably at a solvent-facing surface, not the active site. However, the region in mutant SOD1 related to high affinity for Cu has not been decided.

Objectives: To clarify the role of cysteine 111 residue in mutant human SOD1 by Cu-IMAC and assess the toxicity of each mutant SOD1 by cell culture system.

Methods: We prepared plasmids containing wild-type, mutant SOD1 (A4V, G85R, G93A) or mutant SOD1 with substitution of serine for cysteine (Cys) at Cys 111 residue (i.e. A4V/C111S, G85R/C111S, G93A/C111S). The homogenate of COS7 cells transfected with each plasmid was applied to the column pre-saturated with $CuCl_2$ and proteins were eluted under an imidazole gradient stepwise (Cu-IMAC). The elution profiles of SOD1 from the Cu-IMAC column were analysed by SDS-PAGE and immunoblotting. To investigate the stability of each mutant SOD1, we carried out native-PAGE and cycloheximide assays. To study the viability of N2a cells transfected with each plasmid, we stained these cells with propidium iodide and analysed by flow cytometry.

Results: All mutant SOD1 with C111S exhibited lower affinity for Cu than that without C111S. C111S substitution accelerated the mobility of mutant SOD1 on native-PAGE and stabilized mutant SOD1 on cycloheximide assays. Furthermore, the viability of N2a cells transfected with mutant SOD1 was restored by C111S substitution.

Conclusions: These results suggest that aberrant copper may directly bind at Cys 111 residue or modification at this residue may cause conformational instability in mutant SOD1, that would be harmful to neuronal cells.

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P121 INVOLVEMENT OF CYS111 IN THE STABILITY OF HUMAN COPPER/ZINC-SUPEROXIDE DISMUTASE

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Background: Copper/zinc-superoxide dismutase (Cu/Zn-SOD)-immunoreactive inclusion bodies in motor neurons are observed in not only familial but also sporadic ALS patients. Since it is a key enzyme that catalyses the disproponation of superoxide radicals into molecular oxygen and hydrogen peroxide, Cu/Zn-SOD is thought to be a major target of oxidative stress. Although oxidative stress induces aggregation in mutant and even wild-type Cu/Zn-SOD, the implication in the pathogenesis of ALS is still unclear. We have previously reported that different conformational changes may occur in Greek key loop (loop VI, residues 102–115 in human Cu/Zn-SOD) between wild-type and FALS mutant SODs during the unfolding process (1). We consider that this loop is highly solvent accessible and easily moveable by various denaturing triggers.

Objectives: In this study, we focused on Cys111 in the Greek key loop to elucidate the role of free cysteine residues on a stability of human Cu/Zn-SOD under oxidative stress.

Methods: We obtained recombinant human 2-mercaptoethanolated Cu/Zn-SOD (2-ME-SOD) from Ube Industries Ltd. MALDI-TOF-mass spectrometry (MS) analyses determined that Cys111 in the 2-ME-SOD is specifically 2-mercaptoethanolated and that additional incubation with 20 mM 2-ME removes the 2-ME from the Cys111. This recovered wild-type Cu/Zn-SOD is denoted as re-SOD. In order to compare the stability between 2-ME-SOD and re-SOD, 1) the effect of heat treatment on the secondary (whole) structure of both SODs was assessed by circular dichroism (CD) measurements; 2) the effect of oxidation on both SODs was assessed by SDS-PAGE and anion exchange column chromatography. In addition, we identified the oxidative modification of Cys111 by HPLC and MALDI-TOF-MS (MS-MS) analyses.

Results: CD measurements revealed that this re-SOD is more sensitive to heat treatment than the 2-ME-SOD. The re-SOD was also more easily oxidized and produced oligomers/aggregates compared with 2-ME-SOD. In addition, we determined that a sulphhydryl group of only Cys111 in the re-SOD, but not in the 2-ME-SOD, is irreversibly oxidized to a sulphinic acid or a sulphonic acid by mild oxidation.

Discussion: Our findings suggest that Cys111 is involved in the propensity for oxidation and aggregation of human Cu/Zn-SOD and that a masking of the Cys111 residue by a thiol specific modifier, such as 2-ME, can stabilize ALS mutant SODs.

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P122 AGRREGATION PROPENSITY OF SOD1 PROTEIN: IMPACT OF CHARGE MUTATIONS

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Over 100 mutations in the human gene encoding the cytosolic homodimeric enzyme Cu/Zn superoxide dismutase (SOD) have been linked to the familial neurodegenerative amyotrophic lateral sclerosis (ALS) syndrome. The key event in ALS seems to be the pathological formation of toxic protein aggregates formed by initially unfolded or partly structured SOD protein mutants. However the molecular mechanism behind the neurotoxic effects by these mutants remains elusive.

In a recent study (1) we have mapped out and compared the folding behavior of 15 SOD mutants, each of them correlated to specific patient survival times, ranging from one year for the most aggressive mutant to 17 years for the most mild one after onset of symptoms. All of these mutations move the folding equilibrium towards the denatured monomer, but their effects differ in magnitude and mechanistic origins. Therefore, the mutations can be divided into different categories with either reducing the stability of the monomer (class 1), the strength of the dimer interface (class 2) or both (class 1+2). In general, there is a strong correlation between reduced stability and reduced mean survival times except in those cases where an alteration of the total net negative charge is involved. With a pI of 4.8–5.1, SOD holds a net negative charge under physiological conditions. If a mutation increases the net negative charge of the protein a slower disease progression is observed. In contrast, mutations leading to a decrease of the net negative charge lead to shorter survival times than expected from protein stability data alone. Of all known ALS associated SOD mutants 26% reduce the magnitude of the net negative charge whereas only 7% produce an increase. Our idea is that ALS responds to a combination of low protein stability and reduced magnitude of the net negative charge. We will here present stability data and aggregation propensity for 10 new net charge altering ALS associated SOD1 mutations to further elucidate the question.

One alternative explanation for the behaviour of these mutants might be their interactions with membranes. Since the apo protein is partly unfolded and monomeric

under reducing conditions, it exposes both its hydrophobic core and the hydrophobic dimer-interface to the solvent. Therefore, membrane interactions might be likely to occur for the reduced apo form of different SOD mutants (2). Preliminary studies already indicate interactions of SOD with charged membrane surfaces, which modulate secondary structures and aggregation behaviour of these mutants in a way quite different from the situation of membrane-free aqueous environment. In particular, the presence of charged membranes seems to trigger aggregation of the A4V mutant already at physiological temperatures, while much higher temperature is required in membrane-free solution.

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P123 ARCHAEAL PROTEASOMES DEGRADE MUTANT SUPEROXIDE DISMUTASE-1 AND REDUCE ITS CELLULAR TOXICITY

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Background: The 20S proteasome is a ubiquitous, barrel-shaped protease complex responsible for most of cellular proteolysis, and its reduced activity is thought to be associated with accumulations of aberrant or misfolded proteins resulting in a number of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), spinal and bulbar muscular atrophy (SBMA), Parkinson's disease, and Alzheimer's disease. The 20S proteasomes of archaebacteria (archaea) are structurally simple and proteolytically powerful and thought to be an evolutionary precursor to eukaryotic proteasomes.

Objectives: To treat familial ALS and other intractable neurodegenerative diseases caused by mutant and modified proteins, which are not properly degraded by endogeneous proteasomes and, thus, prone to toxic gain of function resulting in neurodegeneration.

Methods: We cloned the Mm proteasome alpha- (Accession No.: 1480962) and beta-subunits (Accession No.: 1479036) from genomic DNA of Mm and generated a mutant alpha-subunit lacking amino acids 2–13, delta(2–13) alpha-subunit (delta-alpha). We also generated a mutant beta-subunit with Thr1Cys (mβ1). Thr 1 in the beta-subunit of the archaeal proteasome is essential for proteolysis and Thr 1 mutants lose their proteolytic activities. The experiments were performed in both HEK293 and Neuro2a cells. Transfections were performed using Lipofectamine 2000 (Invitrogen) in the MTS assay, or Effectene Transfection Reagent (Qiagen) in other experiments. To analyse turnover of wild-type and mutant SOD1, cycloheximide and pulse chasing studies were performed. We chose MTS assay to evaluate the toxicities of mutant SOD1 with or without Mm proteasomes.

Result: We successfully reproduced the archaeal proteasome in a functional state in mammalian cells and here we show that the archaeal proteasome effectively accelerated species-specific degradation of mutant superoxide dismutase-1 and the mutant polyglutamine tract-extended androgen receptor, causative proteins of familial ALS and SBMA, respectively, by cycloheximide and pulse chase studies, and reduced the cellular toxicities of these mutant proteins by MTS assay. Furthermore, we demonstrate that archaeal proteasome can also degrade other neurodegenerative disease-associated proteins such as alpha-synuclein and tau.

Discussion: Archaeal proteasomes would open a new therapeutic avenue for diseases in which the toxic-gain of proteins are causative.

P124 ALTERATION OF NEUROFILAMENT PHOSPHORYLATION IN MOTOR NEURONS EXPRESSING MUTANT SUPEROXIDE DISMUTASE 1

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Background: Mutations in the copper/zinc superoxide dismutase (SOD1) gene are known to be responsible for familial amyotrophic lateral sclerosis (ALS). Aberrant accumulations of hyperphosphorylated neurofilaments (NFs) at the perikarya and proximal axons are pathological hallmarks of ALS. There is increasing evidence that the phosphorylation of NFs is involved in the pathogenesis of ALS.

Objectives: We examined the effects of mutant SOD1 on the regulation of neurofilament phosphorylation in a motor neuron-neurofilament hybrid (VSC4.1) cell line.

Methods: Following transfection of human SOD1 cDNA of normal (wild-type) and mutant (G93A), cells were differentiated by dibutryl cAMP (1mM) for 24 h and aphidicolin (0.025 μg/ml) for 48 h. We then treated pharmacologic inhibitors of extracellular signal-regulated kinase (ERK) cascade; PD098059, cyclin-dependent kinase (CDK); Olomucine and glycogen synthase kinase-3 (GSK-3); lithium chloride. Also we identified the viability by MTT assay, NFs morphology by immunofluorescence microscopy and protein levels by Western blotting.

Results: Activities of GSK-3, CDK and ERK did not contribute to the phosphorylation of NFs, but rather significantly increased the state of NF phosphorylation in

motoneuronal cells expressing mutant SOD1. Inhibition of ERK cascade in mutant cells led to the hyperphosphorylation of NFs through a persistent activation of c-jun N-terminal kinase (JNK).

Discussion: We suggest a neuroprotective role of NFs by acting as a phosphorylation sink. The mutant SOD1 may alter the signalling process implicated in the phosphorylation of NFs. Also, activation of JNK might contribute to the aberrant hyperphosphorylation of NFs in ALS.

P125 *IN VITRO* MODELS OF MUTANT SOD1-MEDIATED AXONAL DEGENERATION

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Background: Denervation and distal axonal degeneration begin very early in the hindlimbs of SOD1-G93A mice, long before loss of ventral root axons and lumbar motor neurons (1–3). Protection of the distal axon may therefore represent an important therapeutic target. However, the mechanism by which mutant SOD1 causes axonal degeneration remains unknown. *In vitro* models of mutant SOD1-mediated axonal degeneration are needed in order to better understand this phenomenon and to determine whether protection of the axon can prevent death of the motor neuron.

Objective: To develop organotypic dorsal root ganglion and spinal cord cultures expressing mutant SOD1 protein, that reproduce *in vitro* the axonal degeneration observed in the SOD1-G93A mouse.

Methods: Dorsal root ganglion (DRG) cultures were generated from E15 rats or from P4 SOD1-G93A mice. Rat DRG were infected with one of three lentiviruses: wild-type SOD1, SOD1-G93A, or GFP only (control). Axonal outgrowth was measured over four to six weeks in culture. Additionally, organotypic spinal cord slice cultures were prepared from P7 SOD1-G93A mice. Motor axons were extended from the slice cultures by co-culturing with HEK293 cells secreting the growth factor, pleiotrophin. Axons from both systems were examined for pathologic features using a variety of morphological and immunocytochemical techniques.

Results: Lentivirus-infected rat DRG and DRG from SOD1-G93A mice both extended long axons that did not differ in length or morphology from control DRG when followed for four to six weeks *in vitro.* Mutant SOD1 expression throughout the axon was verified by immunocytochemistry. Motor axons were extended from both mutant and wild-type spinal cord cultures, and followed over four weeks *in vitro*. Mutant SOD1 motor neurons and axons were positive for phosphorylated neurofilament, and axons exhibited prominent, neurofilament-positive swellings.

Discussion: We have established organotypic models of sensory and motor axons that will be used to investigate the axonal pathology associated with mutant SOD1. To date, simply expressing mutant SOD1 in cultured axons does not appear to be sufficient to cause axonal degeneration. However, we have not yet tested whether mutant SOD1 axons may be more susceptible to environmental perturbation, such as oxidative stress. In this system we can also test whether the toxicity of mutant SOD1 is due to expression in the axon itself (cell autonomous), or may be associated with expression in non-neuronal cells such as Schwann cells (non-cell-autonomous). Experiments are currently underway to address these hypotheses.

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P126 GENE EXPRESSION IN G93A-SOD1/BV2 CELLS

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Background: Gene expression studies of mutant SOD1 mice in our laboratory have shown that in this animal model of ALS activity, many genes associated with microglia are differentially regulated. These results, together with those of other studies, suggest that neuroinflammation may be an early event in ALS.

Objectives: To obtain more insight into the role of microglia in ALS, we analysed expression of the same panel of genes in BV-2 cells, a microglia cell line, that were transfected with a mutant SOD1 gene, G93A-SOD1.

Methods: Both non-transfected BV-2 cells and BV-2 cells transfected with the wild-type human SOD1 gene served as controls. We then made three types of comparisons of gene expression patterns: non-transfected vs. wild-type transfected; mutant vs. non-transfected; and mutant vs. wild-type transfected.

Results: Significant changes (up- or down-regulation) were observed between mutant and wild-type-transfected or non-transfected for several classes of genes, including those involved in oxidative stress neurotoxicity, neuroinflammation, zinc regulation and apoptosis, as well as in cell markers for microglia. However, changes were also observed when wild-type transfected cells were compared with untransfected cells.

Conclusions: Overall, our results indicate that *in vivo* changes in expression in microglia related genes involve cell types other than microglia, i.e. other cells affect

microglia activation in ALS, and genes of these other cells may also be differently regulated.

P127 ROLE OF ASTROCYTES IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Astrocytes represent the largest glial population in the central nervous system (CNS). They provide metabolic and trophic support to neurons, modulate the microenvironment regulating ion homeostasis, produce a variety of soluble and membrane-associated growth factors, contribute to the formation of the blood-brain barrier (BBB) and interact with endothelial cells. After damage to the CNS, glia are thought to support neural growth and metabolism and scavenge agents toxic to neurons. In particular, neurotoxic events in the CNS are often characterized by an early and pronounced activation of astrocytes and microglia. These cells maintain normal functioning of the nervous system both controlling the extracellular environment and supplying metabolites and growth factors. Preservation of the normal relationship between neurons and glia is critical to maintain normal nervous system function.

Since a strong glial reaction surrounds motor neurons in amyotrophic lateral sclerosis (ALS) patients, recent studies suggested the involvement of reactive astrocytes in the pathogenesis of ALS. Astrocytes, together with endothelial cells, play a key role in BBB, acting as an intermediary between blood and neurons.

Objectives: In order to better understand the role of glial cells in neurodegeneration processes in ALS, we studied the effect of ALS patients' sera on cultured astrocytes.

Methods: Primary cultures of human foetal astrocytes were treated with sera from ALS patients (or healthy donors as controls). Cell response was evaluated by expression levels of different immunoregulatory molecules: TNF-α, IL-1 α and IL-1 β, IL-6, IL-10, RANTES, CD137 (belonging to the family of TNF receptors) and inducible nitric oxide synthase (iNOS).

Results: Our results showed an increase of pro-inflammatory molecules, according to different disease severity. A significant increase of chemokine RANTES (CCL5) was observed in all samples, suggesting a recruitment of T-lymphocytes and macrophages Moreover, some sera induced toxicity and cell death in cultured astrocytes.

Discussion: These findings, although preliminary, suggest that astrocytes may be activated by ALS patient sera, supporting therefore the hypothesis that these cells might play a role in ALS pathogenesis.

P128 LONG TERM CULTURE OF ADULT RAT SPINAL CORD CELLS IN A NOVEL SERUM-FREE MEDIUM AND ON A SYNTHETIC ORGANOSILANE SUBSTRATE: MORPHOLOGICAL, IMMUNOCYTOCHEMICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION

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Background: Culturing adult mammalian spinal cord neurons is a challenging problem due to the inability of the adult neurons to survive in culture after undergoing the trauma of dissection. Hence, most of the cell culture studies have been performed using embryonic spinal cord tissue because embryonic neurons survive better in cell culture. Thus although the embryonic cell culture model is popular, it is not the most appropriate model to study age related disorders, injuries and degenerative diseases of the spinal cord. An adult spinal cord culture model could address this problem.

In this study, we have developed such a model system using a synthetic organosilane substrate and a novel serum-free medium (1) to culture the neurons and the glial cells of the adult rat spinal cord. We have characterized the adult neurons and glial cells morphologically, immunocytochemically and electrophysiologically.

Objectives: 1) Preparing an organosilane substrate to grow the cells; 2) Developing cell isolation process from adult rat spinal cord; 3) Developing serum-free medium to grow the cells; and 4) Immunocytochemical and electrophysiological characterization.

Methods: Cell culture, surface chemistry, tissue preparation, immunocytochemistry, patch-clamp electrophysiology.

Results: In this work, we documented by morphological analysis, immunocytochemistry and electrophysiology, the development of a culture system that promotes the growth and long-term survival of dissociated adult rat spinal cord neurons and glial cells. This system comprises a patternable, non-biological, cell growth promoting organosilane substrate N-1[3-(trimethoxysilyl)propyl]-diethylenetriamine (DETA), coated on a glass surface and an empirically derived novel serum-free medium, supplemented with specific growth factors (1). The culture consisted of 60% neurons and 40% glia.

Neurons were characterized by immunoreactivity for neurofilament150, MAP-2, ISLET-1, choline acetyltransferase (ChAT) and MO-1 antibodies. The glial cells were characterized by immunoreactivity for the GFAP antibody. Electrophysiology experiments indicated that 60% of the cells expressed voltage dependent inward and outward currents similar to neurons and were able to generate single, double and multiple (repetitive firing)

action potentials. Forty per cent of the cells either showed inward, outward or very small inward and huge outward currents and these recordings are similar to the recording documented for the glial cells in the literature. The cultures were maintained for 8–10 weeks.

Discussion and conclusions: These are the first studies to demonstrate that adult rat spinal cord cells can be cultured in a completely defined serum-free medium and on a synthetic silane substrate. This culture system will be a useful tool to study adult mammalian spinal neuron repair, myelination and degeneration, as well as to screen different novel and putative drug candidates for spinal cord repair and degenerative diseases of the spinal cord.

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P129 AMPA RECEPTORS IN CORTICAL NEURONS ARE ALTERED IN A TRANSGENIC MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: There are many evidences implicating glutamatergic toxicity as a contributing factor in the selective neuronal injury occurring in amyotrophic lateral sclerosis (ALS). In spinal motor neurons it has been observed that in a mouse model of ALS the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is almost three-fold more permeable to Na^+ and K^+ than the control whereas the Ca^{2+} permeability does not differ. In addition, altered motor cortex excitability has been reported in ALS patients and preliminary results from our laboratory have indicated an intrinsic cortical neuron hyperexcitability in the G93A mouse model of ALS.

Objectives: The aim of this study is to establish if AMPA receptors of cortical neurons in a model of a familial form of ALS, expressing high levels of the human mutated protein Cu/Zn superoxide dismutase (Gly^{93}→Ala, G93A) present altered biophysical properties.

Methods: We have investigated the electrophysiological properties of AMPA receptors in cortical neurons in culture of non-transgenic mice (control), of transgenic mice expressing high levels of the human wild-type protein (SOD1) and of G93A mice, using the whole cell configuration of the patch-clamp recording technique.

Kainate-induced currents were measured from eight to 14 days *in vitro*. Spectral density analysis of steady-state current noise evoked by kainate agonist and the calculation of single channel conductance has also been performed in the three types of neurons. After plotting variance versus current, the data was fitted with the equation: $v_{ar}(t)=iI(t)-I(t)^2/N$, where i is the single channel current, $I(t)$ is the mean macroscopic current for a given data segment, and N is the number of channels. The maximal P_{open} of the channels underlying each kainate-evoked response was then calculated as $P_{open}=I_{SS}/Ni$, where I_{SS} is the steady-state kainate evoked current. Finally, the single channel conductance was calculated by $\gamma=i/V_M$, where V_M is the membrane potential.

Results: The electrophysiological experiments indicate that the single cell current density and the reversal potential were not modified in G93A cortical neurons compared to control and SOD1. On the contrary, by the spectral density analysis, preliminary data appear to indicate changed values of single channel conductance in the three different cell populations. In fact, the G93A cortical neurons appear to express a different number of receptors with a different single channel current (i) compared to control and SOD1 neurons.

Conclusions: These results show that the AMPA receptors in the three different populations of cortical neurons have different biophysical properties. Since the receptor's permeability and kinetic properties strictly depend on the subunit composition, these results indirectly suggest that the AMPA receptor subunit composition is altered in cortical neurons with the G93A mutant SOD1. As yet it is unknown what significance the altered expression of AMPA receptors has in relation to the ALS pathology. Further experiments are necessary to study the connection between them.

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P130 IVERMECTIN INHIBITS AMPA RECEPTOR-MEDIATED EXCITOTOXICITY IN CULTURED MOTOR NEURONS AND EXTENDS THE LIFE SPAN OF A TRANSGENIC MOUSE MODEL OF ALS

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Background: Excitotoxicity mediated by AMPA receptors contributes to selective motor neuron death in ALS.

Objectives: We wanted to determine the effect of P2 receptor-influencing substances on AMPA receptor-mediated motor neuron death and on the survival of SOD1 (G93A)-mice.

Methods: We studied the selective vulnerability of motor neurons to AMPA receptor-mediated excitotoxicity *in vitro* using purified motor neurons, grown on a pre-established glial feeder layer. After one week in culture they were exposed for 30 min to 300 μM kainate in a modified Krebs

solution. The survival of motor neurons was quantified by counting them in a marked area under phase contrast before pre-incubation, before the exposure and 24 h after the exposure.

To further investigate the relevance of the *in vitro* findings for ALS, we treated SOD1 (G93A)-mice with ivermectin. Treatment was started at 50 days of age and continued until time of death. At 120 days randomly selected control and ivermectin-treated mice were killed for pathological evaluation of neuronal loss in the lumbar spinal cord and of axon loss in the ventral roots.

Results: PPADS, a non-specific P2 receptor antagonist, did not have a significant effect on the motor neuron death after a short exposure to 300 μM kainate. Because the $P2X_4$ receptor, a subtype widely expressed in the brain, is insensitive to PPADS, we also tested ivermectin and cibacron blue 3G-A, allosteric modulators of this receptor subtype. After preincubation of the motor neuron cultures with these substances a complete protection against AMPA-mediated excitotoxicity was seen. The effect was indirect because addition during kainate stimulation alone had no effect, and the number and Ca^{2+}-permeability of the AMPA receptors were not influenced by preincubation with ivermectin. Preincubation with low concentrations of ATP (100 μM), the natural agonist of the $P2X_4$ receptor, also protected the motor neurons against a subsequent excitotoxic stimulation, while higher concentrations of ATP (1mM) were toxic. Ivermectin increased the toxicity of low ATP concentrations, indicating that ivermectin can potentiate the effect of ATP on its receptor. To further investigate the relevance of these findings for ALS, we treated SOD1 (G93A)-mice with ivermectin. This resulted in an extension of the life span of these transgenic animals by 9.1%. This protective effect was confirmed by histological evaluation of the lumbar spinal cord (65% increase of neurons/section for ivermectin-treated animals) and of the ventral roots (60% increase of area occupied by axons in the ivermectin-treated animals).

Conclusions: We demonstrated that ivermectin induced a protective mechanism in motor neurons, *in vivo* and *in vitro*, against excitotoxic assaults. Our *in vitro* data suggest that this is due to the interaction of ivermectin with ATP and the $P2X_4$ receptor.

P131 AMPA RECEPTORS AS A TARGET FOR PHARMACOLOGICAL NEUROPROTECTION: MOLECULAR MECHANSIMS

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Background: The term excitotoxicity was coined for the overstimulation of neurons via glutamate receptor channels. AMPA-type glutamate receptors (AMPA-R) are the most abundant excitatory transmitter receptors of the central nervous system. They play a major role in the cascade of cell damage in neurodegenerative diseases such as amyotrophic lateral sclerosis. Neurons that are vulnerable to excitotoxicity express specific subtypes of AMPA-R as defined by the combination of subunits, by alternative splicing, and by nuclear editing of subunit mRNAs. The investigation of the neurophysiological properties of specific AMPA-R allows for the detection of factors of vulnerability on the molecular level.

Objectives: To define molecular targets for pharmacological neuroprotection via modulation of the synaptic input to motor neurons and to test pharmacological effects of candidate compounds at the cellular and molecular level.

Methods: We combined in situ hybridization techniques with immunohistochemistry to reveal the specific receptor pattern of vulnerable neurons. In our neurophysiological studies, we combined the characterization of AMPA-R subtypes that were recombinantly expressed with the analysis of native receptors at the single channel level. Synaptic currents, networks of cultured motor neurons and the brain slice technique were studied by patch-clamp and calcium imaging techniques. The data from these studies gave a basis for the testing of compounds that are candidates for pharmacological neuroprotection.

Results: We investigated the molecular mechanism of action of a broad variety of compounds interacting with AMPA-R (e.g. RPR119990, RPR117824, memantine, topiramate, IEM1460, ZK187638, ZK200775, derivates of valproic acid). The receptor channel subtype specificity of the compounds was characterized as well as the molecular mechanism of action on the single channel and on the synaptic level. Competitive and non-competitive block mechanisms were observed. The molecular drug profiles were compared with data from synaptic recordings and calcium imaging to allow for predictions of the *in vivo* mechanisms of action.

Discussion: *In vitro* and *in vivo* models of neurodegeneration are used to develop more effective strategies for pharmacological neuroprotection. Antagonizing glutamatergic neurotransmission may be a therapeutic strategy in neurodegenerative diseases. The investigation of the synaptic integration of vulnerable neurons and of the molecular mechanisms of pharmacological AMPA-R activation and modulation is crucially required to reveal pharmacological strategies that specifically interfere with the pathophysiological cascade of excitotoxicity.

Acknowledgement: This study was supported by the DFG, the DGM, Sanofi-Aventis, and Schering.

P132 ANTI-APOPTOTIC ACTIVITY MAINTENANCE OF BRAIN DERIVED NEUROTROPHIC FACTOR AND THE C FRAGMENT OF THE TETANUS TOXIN FUSION PROTEINS

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Background: Neurotrophic factors have been widely suggested as a treatment for multiple diseases including motor neuron pathologies, such as amyotrophic lateral sclerosis (ALS). However, clinical trials in which growth factors have been systematically administered to ALS patients have not been effective, owing in part to the short half-life of these factors and their low concentrations at target sites. A possible treatment strategy is the use of the atoxic C fragment of the tetanus toxin (TTC) as a neurotrophic factor carrier to the motor neurons. However, in this case, the activity of trophic factors should be tested because their fusion to proteins could alter their folding and conformation, thus undermining their neuroprotective properties.

Objectives: We explored the brain derived neurotrophic factor (BDNF) activity maintenance after genetic fusion with the C fragment of the tetanus toxin (BDNF-TTC).

Methods: Recombinant proteins (TTC, BDNF-TTC and BDNF) were purified from *Escherichia coli* strain Bl21-C43. Eluted proteins were evaluated by Coomassie blue staining and Western blot analysis. Apoptosis in mouse neuroblastoma Neuro 2A cells (2.5×10^5) was induced by serum deprivation for 48 and 72 h. Recombinant proteins were added at doses of 10, 50 and 250 ng/ml culture. Quantification of apoptosis was performed using an Annexin V staining protocol to determine the exposure of phosphatidylserine in the cell surface. Akt activation assay was developed with cultured primary cortical neurons incubated with recombinant proteins for 1 h at 37°C. Fluorescence intensity from activated Akt was measured by confocal microscopy.

Results: The functionality of BDNF and BDNF-TTC proteins was assessed analysing the effect of these proteins in the inhibition of apoptosis induced in Neuro2A cells by serum deprivation. Forty-eight hour treatment with BDNF showed significant differences from the control for all the doses studied. The other proteins did not show any effect at this time although BDNF-TTC displayed a tendency for apoptosis inhibition, but this difference was not statistically significant. The three recombinant proteins (BDNF, BDNF-TTC and TTC) were able to inhibit apoptosis after serum deprivation in Neuro2A after 72 h. Immunodetection of Akt in cultured primary cortical neurons, after incubation with the different proteins, displayed evident differences of signal between the cultures treated with the three proteins and the control. The strongest AKT signal was observed with BDNF, followed by the fusion protein and finally with TTC alone.

Conclusion: We demonstrated that BDNF fused with TTC induces the neuronal survival Akt kinase pathway in cortical culture neurons and maintains its previously described antiapoptotic neuronal activity *in vitro.*

Acknowledgement: This work was supported by Instituto de Salud Carlos III-Fondo de Investigación Sanitaria (PI020840).

P133 EFFECTS OF ACTIVATED PROTEIN C ON INSULIN-LIKE GROWTH FACTOR (IGF-I), IGF-I RECEPTOR AND AKT, AND ITS NEUROPROTECTIVE EFFECTS AFTER SPINAL CORD ISCHAEMIA

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Background and purpose: Activated protein C (APC) has beneficial effects on ischemia reperfusion injury in neurons. However, the possible mechanism of such beneficial effect is not fully understood. The aim of this study was to investigate the effects and possible mechanisms of APC on ischemic spinal cord damage.

Methods: After induction of spinal cord ischemia, APC (group A) or vehicle (group I) was injected intravenously. Severity of ischemic damage was analysed by counting the number of motor neurons. To investigate the mechanisms by which APC prevents ischemic spinal cord damage, we performed immunoreactivity and Western blotting of insulin-like growth factor 1 (IGF-1), IGF-1 receptor and phosphorylated serine-threonine kinase (p-Akt).

Results: APC eased the functional deficits and increased the number of motor neurons after ischemia. Immunoreactivity of IGF-1 in group A was stronger than in group I at 8 h after reperfusion but was at the same level at 1 day. Induction of IGF-1 receptor and the downstream factor p-Akt was stronger and more prolonged in group A.

Conclusions: These results indicate that induction of IGF-1, IGF-1 receptor and p-Akt might partially explain the neuroprotective effects of APC after transient spinal cord ischemia in rabbit.

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THEME 8 GENETICS AND EPIDEMIOLOGY

P134 POSITIONAL CLONING OF A NOVEL GENE FOR PROGRESSIVE MOTOR NEURON DEGENERATION

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Background: Motor neuron disease and hereditary motor neuropathies (HMN) are related neurodegenerative diseases that cause the selective progressive death of motor neurons. Historically, the disorders have been classified separately based upon rate of disease progression, with MND/ALS usually rapidly progressive and fatal, while HMN is slowly progressive and non-fatal. However, the HMNs and MNDs share clinical and pathological features 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. Genes identified from HMN and MND/ALS families have also been associated with disease in sporadic MND/ALS cases.

Objectives: Our aim is to use linkage analysis and positional cloning approaches to identify new disease genes for motor neuron degeneration and establish whether these are more broadly involved in familial and sporadic motor neuron degeneration, as pathogenic or susceptibility genes.

Methods: We performed a genome scan for linkage in a large HMN family comprising 25 individuals including 12 affected. A transcript map of the disease locus was generated using the human genome annotation projects of the Sanger Centre (Ensembl) and UCSC (Genome Browser). Candidate genes were selected based upon known or inferred function and expression. Mutation analysis was performed by high resolution melting (LightScanner, Idaho Technology) and direct sequencing.

Results: A genome-wide linkage scan identified a novel HMN locus on chromosome 7q34-q36. Significant evidence for linkage was obtained for D7S2546 with a two-point lod score of 3.33. A multipoint lod score of 3.59 was obtained at the same marker. Additional support for linkage was obtained for two adjacent markers with multipoint lod scores of 3.50 and 3.44. Recombinant haplotype analysis defined a 21.8 cM candidate interval. We generated a transcript map of the candidate region that spans 13 Mb and contains 247 entries for known or putative protein-coding genes. The interval encompasses large clusters of genes encoding T-cell receptors (86), olfactory receptors (27), and taste receptors (9) as well as trypsin molecules (10). The remaining 115 transcripts were inspected to identify any obvious candidate genes based upon our knowledge of previously implicated HMN/MND genes or disease mechanisms. Candidate disease genes are currently being screened for mutations.

Discussion: We have identified a locus for progressive motor neuron degeneration on chromosome 7q34-q36. Work is underway to positionally clone the gene in question. Once identified, we will investigate HMN, FALS, and sporadic MND/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.

P135 SYSTEMATIC ELIMINATION OF PREVALENT MYELIN SEQUENCES FROM MOUSE SPINAL CORD cDNA PREPARATIONS

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Background: We have previously demonstrated by suppression subtractive hybridization (SSH) and mirror orientation selection (MOS) that a large portion of sequences differentiating spinal cord from visual cortex in the mouse can be attributed to cDNA derived from myelin. Fully half of all up-regulated sequences in the spinal cord subtracted against visual cortex are of glial cell origin. Proteolipid protein alone accounts for some 40% of all up-regulated sequences in the spinal cord. It is possible, although unlikely, that these differences reflect increased numbers of glial cells in this structure or possibly the ongoing metabolic activity of those cells normally present; however, it is preferable to study the expression of mRNA in motor neurons unobstructed by the overwhelming presence of glial derived sequences.

Methods: Two approaches were designed to reduce the number of glial derived sequences in subtraction preparations before proceeding with SSH. In the first procedure, we have attached biotinylated oligonucleotides of known myelin sequences to streptavidin coated magnetic beads and, by incubating cDNA preparations from spinal cord and visual cortex to these beads with the attached capture sequences, are subtracting out specific prevalent cDNAs. In the second procedure, myelin rich cDNA from brain white matter was used as a driver for the subtraction to reduce myelin sequences present in the spinal cord.

Results: Taking the sequence derived from proteolipid protein (plp), which represents the most abundant myelin sequence up-regulated in the spinal cord when subtracted against the visual cortex, as an example: the occurrence of this sequence was reduced by 1000-fold by semiquantitive polymer chain reaction before and after bead subtraction from spinal cord and visual cortex. When

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this pre-subtracted cDNA was subsequently used to perform SSH between spinal cortex and visual cortex, the final results showed a consistent 90% reduction of plp sequences. In the alternate procedure, where spinal cord was subtracted directly against cDNA preparations derived from white matter, we have observed no sequences that can be positively identified as originating from glial cells.

Discussion: There can be little doubt that prevalent myelin derived sequences present in spinal cord and visual cortex can be reduced precipitously by pre-subtracting the original cDNA preparations against immobilized biotin labelled myelin sequences with streptavidin beads as well as subtracting spinal cord directly against myelin derived cDNA sequences. This will allow the removal of the glial derived cDNA sequences as well as other abundant sequences originating in the nerve cells in the spinal cord and visual cortex themselves, and provide a more in-depth analysis of any differentially expressed genes in these structures. It is anticipated that analyses of these final motor neuron derived sequences will lend some clue as to the susceptibility of motor neurons in motor neuron disease among other pathologies affecting these cells.

P136 ALS6 REVISITED: LINKAGE ANALYSIS WITH DNA MICROARRAYS

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Background: Gene hunting in familial ALS (FALS) is a difficult task due to rapid disease progression and difficulty in collecting DNA for linkage analysis. We have conducted work on a number of families with ALS using Affymetrix 10K GeneChip® DNA microarrays. These allow simultaneous genotyping of over 10,000 single nucleotide polymorphisms (SNPs). Subsequent analysis of the SNP calls results in rapid determination of regions of potential linkage.

Objectives: To identify the disease-causing gene in a family with aggressive autosomal dominant ALS (ALS 6). A previous genome-wide study using microsatellite markers had demonstrated strong linkage only to chromosome 16q (maximum multipoint log of the odds (LOD) score of 3.85) and a haplotype that segregated with disease.

Methods: DNA from five affected individuals was processed according to the Affymetrix protocol before being hybridized onto GeneChips®. Following staining and washing in a fluidics station, chips were then scanned. The output was formatted for linkage analysis using the Merlin linkage package. Regions of potential linkage were then fine-mapped using microsatellite markers. Haplotype analysis was conducted to determine phase and segregation with disease.

Results: The previously linked region on chromosome 16 was verified in this study. However, regions on chromosomes 2, 9 and 11 also demonstrated significant LOD scores. Fine mapping and haplotype analysis excluded the regions on chromosomes 2 and 11, but a consistent haplotype segregating with disease was found at 9q31 (maximum LOD score of 2.3).

Discussion: Although DNA microarrays dramatically reduce the time and labour requirement for linkage analysis there are a number of inherent problems. The vast amount of data that needs to be analysed requires great computer power. This can be a significant problem when analysing large kindreds.

The discovery of two or more loci that are potentially linked with disease is a problem that is likely to be increasingly experienced as DNA microarrays become the standard approach for linkage analysis. The higher resolution they provide can pick up smaller areas that would previously have been missed by microsatellite marker sets. This can lead to an increase in the false positive rate and the problem of not knowing which region to investigate further.

P137 SCREENING FOR GENETIC VARIATION IN SEX HORMONE RECEPTOR GENES IN A DUTCH POPULATION

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Background: ALS predominates in males, although this sexual dimorphism diminishes with age. Women develop ALS at an older age than men and have an earlier menopause compared to controls. These features suggest a role for sex hormones in the pathogenesis of ALS. Furthermore, both *in vitro* and *in vivo* studies have shown that oestrogen and androgens (testosterone and dihydrotestosterone) protect neurons from degeneration. The neuroprotective actions of these steroid hormones are mostly receptor-mediated.

Objectives: To determine whether genetic variation in the coding regions of the androgen receptor (AR) and the oestrogen receptor (ER) alpha and beta are genetic susceptibility factors in ALS.

Methods: Genomic DNA from a large set of Dutch well defined ALS patients and controls was extracted from blood samples. The coding region and untranslated regions of the AR, ERα and ERβ were amplified by PCR and products were re-sequenced to detect (novel) mutations.

Subjects were further genotyped for all known SNPs using allelic discrimination with TaqMan assays.

Allele frequencies were determined and tested for association.

Results: No novel mutations were detected in the coding and untranslated regions of the three genes. Genotyping

the known polymorphisms yielded no direct association with ALS. However, we detected a lower incidence of a SNP in exon 8 of ERα in ALS patients with bulbar onset ($p=0.02$).

Discussion and conclusions: We identified a relationship between an ERα single nucleotide polymorphism and bulbar ALS, suggesting a protective effect of this SNP. Our results will be validated in a larger population. Furthermore, the biological consequences of this SNP will be investigated.

P138 ADULT-ONSET PRIMARY LATERAL SCLEROSIS IS NOT ASSOCIATED WITH MUTATIONS IN THE ALS2 GENE

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Background: PLS is a diagnosis of exclusion in patients with an adult onset gradually progressive pure upper motor neuron (UMN) syndrome and could be part of the clinical spectrum of ALS. Adult onset PLS is almost always sporadic. Recessive mutations in ALS2 are causative for early onset upper motor neuron diseases, including infantile ascending hereditary spastic paralysis (IAHSP), juvenile amyotrophic lateral sclerosis (ALS2) and juvenile primary lateral sclerosis (JPLS).

Objective: To assess the role of ALS2 in adult onset PLS.

Methods: We screened a population of 51 adult onset PLS patients by DHPLC methods. Thirty-nine patients had disease onset in the legs, nine in the bulbar region and three in the arms.

Results: Fourteen sequence variants were detected within ALS2, of which 12 were known single nucleotide polymorphisms (SNPs) and two were new sequence variants without pathogenic significance.

Conclusion: These results indicate that mutations of ALS2 are not a common cause of adult onset PLS.

P139 ARRAY CGH OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: NEW CANDIDATE GENES

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Background: Amyotrophic lateral sclerosis (ALS) is a devastating disorder of the central nervous system that leads to progressive loss of upper and lower motor neurons.

Methods: We have used comparative genomic hybridisation array (CGH array) to screen patients with definite ALS for cryptic chromosomal aberrations in the whole genome.

Results: In 113 patients, we detected several deletions and duplications. Most interestingly, two patients showed duplications at the site of the *KIF25* gene on chromosome 6q27, one patient had an alteration on chromosome 6p21.2 (heavy chain dynein polypeptide, DNAH8), and a fourth had an augmentation on chromosome 17q25.1 (intermediate dynein polypeptide, DNAI2). We also found a duplication on chromosome 1p32.2, close to the gene of the glutamate transporter EAAT5. There were several other alterations, consisting of genes of unknown function; however, their total number was not increased if compared with non-neurological, age-matched controls.

Conclusions: Among 113 patients with ALS, the CGH array technique was able to detect four submicroscopic chromosomal alterations in three genes relevant for motor protein function. We consider these genes candidates for ALS and related disorders. We did not find evidence for increased genomic instability in ALS.

P140 LARGE-SCALE GENOME ASSOCIATION STUDY FOR AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a heterogeneous group of neurodegenerative disorders characterized by progressive wasting and weakness of limb, bulbar and respiratory muscles due to the selective degeneration of motor neurons in the brain and spinal cord. Most cases (90%) of ALS are sporadic. To date, several hypotheses for mechanisms of selective motor neuron death, including glutamate-mediated excitotoxicity, oxidative stress, cytoskeleton abnormalities, and autoimmunity, have been put forward. However, the pathogenesis of ALS remains largely unknown.

Objective: To identify genes associated with susceptibility to ALS, we performed a large-scale case-control study by means of single nucleotide polymorphisms (SNPs) in Japanese patients with ALS.

Subjects and methods: We obtained samples from 611 ALS cases as a part of the Japanese project of Personalized Medicine (BioBank Japan) with written informed consent. DNAs were prepared from each blood sample using standard protocols. The study was approved by the Institutional Review and Ethics Boards of the University of Tokyo and RIKEN Yokohama Institute. The SNPs used in this study were selected from the IMS-JST

Japanese SNP database. We employed the Invader assay combined with high-throughput multiplex PCR to screen the disease associated genomic regions.

Results: We first genotyped 94 ALS patients at 50,368 SNPs and compared their allelic or genotype frequencies at these loci with those in the Japanese general population. All SNPs were successfully genotyped, and 1033 SNPs showed p-value of 0.01 or less. We further genotyped the SNPs that had p values less than 0.01 in a larger replication panel of individuals with ALS. Finally, we found that a total of three SNPs in two unrelated genes were strongly associated with ALS ($p < 0.0001$). We have now constructed high-density SNP maps around these marker SNPs.

Discussion: In this study, we performed a large-scale association study to identify the genes associated with ALS. Methodologically, our screening system has at least two advantages. First, since all SNPs used in this study were isolated around gene regions, we can efficiently investigate SNPs that are associated with susceptibility to common diseases. Secondly, using the Invader assay combined with multiplex PCR, our method allowed us low-cost screening that requires as little as 0.1 ng of genomic DNA per single SNP. Here we identified three candidate SNPs in the two unrelated genes. Both genetic and functional analyses of these candidate genes are now ongoing.

P141 D90A SOD1 MUTATION FOUND IN A SPORADIC ALS PATIENT WITH HOMOZYGOSIS AND RECESSIVE INHERITANCE

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Background: Autosomal dominant inheritance accounts for some 10% of cases of amyotrophic lateral sclerosis (ALS). A subgroup of these familial cases (around 20% of them) is linked to mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) (1). One of these mutations, D90A is the only one known to date that is consistent with both autosomal dominant and recessive inheritance (typically found in the Scandinavian population) (2).

Objective: To study the genetic characteristics of patients in both sporadic and familial ALS cases from the Spanish population.

Methods: The presence of mutations in the SOD1 gene was screened for in patients with probable or definite ALS. Methods included single strand conformational shift polymorphism (SSCP) analysis and direct sequencing. To confirm the mutation we used a PCR-RFLP approach (2).

DNA from relatives (parents, uncles and aunts, sister and spouse) were also analysed to pinpoint the inheritance pattern of the trait.

Results: Molecular analysis showed that one patient was homozygous for the D90A mutation. The parents and the sister were asymptomatic carriers for the same mutation. The proband had onset of weakness in legs and developed progressive tetraparesis in four years. Clinical examination revealed bilateral pyramidal signs and motor neuron involvement in the arms and legs. He failed to show bulbar or respiratory symptoms.

Discussion and conclusions: Our group documents the first case in the Spanish population of a patient with ALS associated with a homozygous D90A SOD1 mutation. This is one of the few families reported outside the Scandinavian population with the D90A SOD1 mutation associated with ALS. Current studies are trying to identify a protective factor in families with the typical recessive inheritance (3,4).

Our data could help to address studies on this protective genetic factor, since the family presented here belongs to a different ethnic background.

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P142 SCREENING OF SUPEROXIDE DISMUTASE 1 GENE MUTATIONS IN CHINESE PATIENTS WITH FAMILIAL AND SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder involving motor neurons in the motor cortex, brainstem and spinal cord. Ninety per cent of cases 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 the 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. However, only a few familial cases and no sporadic case with SOD1 gene mutations, have been reported in China

Objectives: We performed a retrospective study of the distribution of SOD1 gene mutations in a large cohort of Chinese ALS patients in order to assess the frequency of SOD1 gene mutations in FALS and SALS cases. Clinical features of patients with SOD1 mutations are reported.

Methods: Blood was collected from 18 individuals from two ALS families and 63 patients with sporadic ALS. 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 pre-mix kit.

Results: In five out of 16 individuals from one family we found a novel missense mutation in the SOD1 gene, which was heterozygous for the mutation, GAA to GTA, causing the substitution of valine for glutamic acid at codon 133 (Glu133Val) in exon 5. The proband has a rapid progressive disease course. The heterozygous G72C mutation was found in a suffering 'sporadic' son and his healthy father. Clinically, the patient exhibited early onset and rapid disease progression. According to the gene test result and the complementary family history, we identified this as an ALS family. In two out of 63 SALS patients we found two novel missense mutations. A new V29A missense mutation in exon 2 was found in a sporadic patient with a typical progressive disease course. A new N86I missense mutation in exon 4 was found in a sporadic patient with a rapid progressive disease course.

Discussion and conclusions: This study firstly reports screening of gene mutations in SOD1 in Chinese sporadic ALS patients. The frequency of SOD1 gene mutations (3.1%) in SALS cases was comparable with that found in other surveys in different cohorts all over the world. Three new mutations were found in this study.

P143 VEGF C2578A POLYMORPHISM DOES NOT CONTRIBUTE TO AMYOTROPHIC LATERAL SCLEROSIS SUSCEPTIBILITY IN SPORADIC CHINESE PATIENTS

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Background and objective: A linkage and association of the VEGF (vascular endothelial growth factor) C2578A polymorphism and amyotrophic lateral sclerosis (ALS) has been found in some studies (1,2). We analysed the C2578A polymorphism in sporadic ALS patients from a Chinese population.

Methods: The polymorphism was analysed in 115 patients and 200 healthy individuals by amplifying across position 2705 to 2494 of the promoter region of the *VEGF* gene.

Results: It was found that the frequency of the allele A was 24% in ALS patients and 28% in healthy individuals ($p=0.264$). Comparing the background of this polymorphism in healthy individuals between Chinese and Caucasians, significant decreases were found in the frequencies of the A/A genotype and allele A ($p<0.001$).

Discussion: We concluded that VEGF C2578A polymorphism did not confer a susceptibility to sporadic Chinese ALS patients, which was in disagreement with that reported previously in Caucasian populations (2) and might be ascribed to the different genetic background between Chinese and Caucasians.

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P144 SCREENING FOR POTENTIAL CANDIDATE GENES AND RISK FACTORS IN A LARGE COHORT OF MND CASES: ASSESSING THE CONTRIBUTION OF ANG, CHMP2B AND HFE

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Background: Although 10% of MND is familial, genetic factors are also thought to play a role in the sporadic disease. Mutations have recently been found in the angiogenin (*ANG*) gene in both familial and sporadic cases (1). In addition, a mutation in chromatin modifying protein 2B (CHMP2B), usually associated with frontotemporal dementia, has been found in two cases of ALS (2).

Recent evidence also supports a role for the hemochromatosis (*HFE*) gene in MND, with the H63D allele showing an increased frequency in MND cases, while there is no association with the C282Y mutation (3,4).

Objectives: 1) To screen ANG and CHMP2B in a large cohort of MND patients for mutations associated with the disease. 2) To determine allele frequencies of the *HFE* gene in the largest cohort of cases to date, compared to healthy controls.

Methods: 1) PCR and direct sequencing of the exon encoding ANG and exons 1–6 of CHMP2B were carried out on 120 DNAs extracted from CNS tissue. In addition,

ANG was screened in a further 400 DNA samples extracted from blood samples.

2) The HFE H63D and C282Y mutations were detected by PCR of exons 2 and 4 followed by restriction enzyme digestion with MboI and RsaI, respectively. Five hundred and twenty MND cases were genotyped and the allele frequencies compared with 400 age-matched healthy controls.

Results: ANG: Out of the first 100 samples sequenced, no mutations have been found, although a previously reported T to G polymorphism has been identified at nt 330.

CHMP2B: Of the 100 samples sequenced, no mutations have been identified in exons 1 or 3, although 2 novel nucleotide substitutions have been found in the 5'UTR, at positions −151 and −102, upstream of the ATG.

HFE: Screening of the H63D mutation has identified the D63 allele to be present at a frequency of 17% in our MND population, while the Y282 allele is present at a frequency of 11%.

Discussion and conclusions: The ANG gene mutations had previously been found in 1% of cases screened, so we would expect several mutations to be identified in our further cases. This suggests ANG is not a common cause of MND.

The CHMP2B gene has only been associated with MND in two cases to date. Screening of all 6 exons in over 100 cases will suggest how frequently changes in this gene are associated with MND

The role of HFE mutations in MND is not understood, although there is increasing evidence of the H63D allele being associated with the disease. This allele is much older in origin than the C282Y allele, which may explain why there is a stronger disease association with the D63 allele than the Y282.

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P145 ELEVATED SERUM ANGIOGENIN IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: An allelic association and a series of novel mutations in the angiogenin gene suggest a role for angiogenin in the pathogenesis of amyotrophic lateral sclerosis (ALS) (1). Deletions in the hypoxia-reponsive promoter of the related peptide vascular endothelial growth factor (VEGF) lead to an ALS phenotype in mice, and altered VEGF levels have been reported in ALS patients (2). However, the patterns of serum angiogenin expression among ALS patients have not previously been assessed.

Objective: The aim of this study was to investigate whether serum angiogenin and VEGF levels were altered in a large cohort of ALS patients.

Patients and methods: Serum angiogenin and VEGF levels were quantified in 79 patients with definite or probable ALS by the El Escorial criteria, and in 72 healthy control subjects. Among ALS patients, 57 presented with spinal onset symptoms and 22 with bulbar symptoms. Blood was drawn at diagnosis, and follow-up 12 month sera were available in 19 cases. Serum levels were determined using a quantitative sandwich enzyme-linked immunoassay.

Results: ALS patients exhibited significantly higher serum angiogenin ($p=0.006$) but not VEGF ($p=0.55$) compared to control subjects. Comparison between subgroups showed significant elevation in angiogenin for spinal ($p<0.001$) but not bulbar ($p=0.11$) onset ALS. Angiogenin levels at 12 months did not differ from baseline. No correlation was noted between angiogenin and VEGF levels ($r=-0.08$, $p=0.49$) in ALS patient serum.

Conclusions: These data suggest a modest elevation in serum ANG in ALS. Further investigation will be required to assess the utility of serum ANG as a biomarker for ALS and as a predictor of disease progression.

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P146 MUTATIONS IN REGULATORY ELEMENTS OF METALLOTHIONEIN ASSOCIATED WITH ALS

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Background: Several lines of evidence suggest that changes in zinc disposition could play a key role in the initiation and/or progression of ALS. Superoxide dismutase-1 (SOD1), mutations in which are a known cause of the disease, binds zinc and mutant forms exhibit altered binding. Metallothioneins (MTs), which bind and regulate levels of cellular zinc, are up-regulated in mutant SOD1

mice, and mutant mice with MT genes deleted reach symptom onset and death sooner than wild-type controls. Alterations in zinc levels are also associated with all the major pathologies observed in ALS, including oxidative stress, glutamate excitotoxicity, neuroinflammation, aggregation, and others.

MTs exist in several different forms, MT-1, 2, and 3 and 4. In humans, MT-1 itself exists in at least seven isoforms of closely related but non-identical sequences. It is not known whether these different isoforms have different functional properties, including zinc binding.

Objectives: We compared the gene sequences of these different MT-1 isoforms, as well as human MT-2 (MT-2A) from ALS patients and controls.

Methods: DNA was isolated from white blood cells or spinal cord tissue, and PCR used to identify and analyse MT sequences in this DNA.

Results: No differences in coding region sequences were detected in any of these MTs, but in one of the MT-1 isoforms, MT-1E, we found a C to G change in the promoter region or 5' UTR region of the gene. The change created a new metal response element (MRE) consensus sequence on both strands of DNA. We are currently investigating whether the frequency of this mutation differs between individuals with ALS and controls.

Conclusions: The MREs present in the promoter region of MTs allow zinc to regulate expression of this zinc-binding protein. Our results suggest that zinc regulation of MT-1 expression may be altered by a single nucleotide mutation in some individuals.

P147 GENOTYPE AND SERUM LEVEL OF INTERLEUKIN-6 IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: The origin of motor neuron degeneration in ALS is still unknown. Experimental studies have shown that local inflammatory reaction plays a role in the pathogenesis of ALS. Interleukin-6 (IL-6) is a major regulator of the acute phase of inflammatory reaction and possesses neurotrophic properties. Animal models and human studies have shown that this cytokine can be involved in the process of neurodegeneration. Genes belonging to the cytokine family were also studied as candidate genes involved in the pathogenesis of SALS.

Objective: To study the G/C polymorphism of IL-6 as well as the serum level of IL-6, which could be potentially involved in the inflammation reaction, in sporadic ALS (SALS) patients compared to controls.

Material and methods: Genotyping of IL-6 was performed in 127 SALS cases with definite or probable diagnosis of ALS according to WFN criteria and 251 healthy controls matched for age (± 3 years) and sex. The polymorphisms were studied using PCR technique and restricted enzyme digestion.

To estimate the serum level of IL-6 we studied 35 SALS patients fulfilling WFN criteria and 16 healthy controls. Subjects were not eligible for the study if there was medical evidence of any infection, inflammatory, immunologic or allergic processes. There was no difference in diet, age and medication between ALS and healthy subjects. Serum was consecutively separated from freshly drawn blood samples, taken in the morning from fasting patients and controls, and was stored at -70°C until assayed. An enzyme-linked immunosorbent assay (ELISA) method with commercially available kit (R&D system) was used for serum IL-6 analysis.

Results: The distribution of genotypes and alleles of IL-6 G/C polymorphism between ALS cases and controls was not significantly different, i.e. genotypes: G/G 42 (33%) vs. 70 (28%), G/C 58(46%) vs. 119 (47%), C/C 27 (21%) vs. 62(25%), respectively, and alleles: G 142 (56%) vs. 259 (52%), C 112 (44%) vs. 243 (48%), respectively.

The mean ($\pm$SD) serum values of IL-6 in ALS and control subjects were: 1.41 ± 1.94 pg/ml vs. 2.76 ± 3.02 pg/ml, respectively. Thus, there was a noticeable decrease of the level of serum IL-6 in ALS patients compared to the controls; however, the difference did not reach statistical significance ($p=0.05$). There was also no correlation between serum IL-6 level and age, gender, duration of the disease, type of onset of the disease, Norris and ALSFRS scales in SALS patients (Pearson correlation).

Conclusions: These data show no correlation between genotype and serum level of IL-6 and increased risk for developing sALS.

P148 LACK OF ASSOCIATION OF P450 1A1/1A2 POLYMORPHISMS WITH SPORADIC ALS AND ABSENCE OF INTERACTION WITH THE PON CLUSTER

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Background: Variation in genes for xenobiotic responsive proteins may modulate susceptibility towards ALS as we recently demonstrated for the paraoxonase (PON) gene cluster (1).

Objectives: To investigate the association and interaction of CYP1A1/A2 gene polymorphisms with sporadic ALS (SALS).

Methods: We investigated the association of three CYP1A1 and CYP1A2 polymorphisms (rs4646421,

rs762551, rs2470890) in a case-control North American Caucasian cohort (n=315/380). Genotyping was performed using TaqMan® SNP Genotyping Assays. Data were analysed using SPSS and Haploview. Gene x gene interactions were carried out using multifactor dimensionality reduction (MDR).

Results: No association with SALS was found with any of the three polymorphisms or their haplotypes. MDR showed a lack of gene x gene interactions of the three polymorphisms with each other and also with the PON cluster associated polymorphisms (rs10487132 and rs6954345) (1).

Conclusions: CYP1A1 and CYP1A2 polymorphisms are not associated with SALS. There is a lack of interaction between PON cluster and the CYP1A1/2 genes.

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P149 SERPINA3 SIGNAL REGION A/T POLYMORPHISM IS A RISK FACTOR OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS) IN A POLISH POPULATION

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Background: Genetic factors involved in the pathogenesis of SALS still remain largely unknown. One candidate gene might be SERPINA3, a serine protease inhibitor. SERPINA3 signal region A/T polymorphism influences SERPINA3 protein expression with the highest levels in the T allele carriers. It has been shown that imbalance of serine proteases and internalized serpins may play a role in the pathogenesis of SALS. Protein complexes formed by inhibitors of serine and cysteine protease system along with damaged Nf proteins, may accumulate within the cell bodies as neuronal inclusions in SALS individuals.

Objective: To study a possible association between SERPINA3 signal region A/T polymorphism and the risk of SALS compared to healthy controls in a Polish population.

Material and methods: We have included 131 patients with SALS and 404 healthy controls matched for age and sex. The definite or probable diagnosis of SALS was established according to El Escorial criteria (1998). Familial ALS (FALS) cases were excluded based on positive ALS family history. The polymorphisms were studied by PCR and restriction enzyme digestion.

Results: The distribution of SERPINA3 signal region A/T polymorphism did not differ between the cases and the controls (cases, n=131: CC −33, 25.1%; CT −64, 48.9%; TT −34, 26.0%; controls, n=404: CC −106, 26.2%, CT −193, 47.8%, TT −105, 26.0%). However, when bulbar and limb sALS onset cases were studied separately, the SERPINA3 A/T polymorphism influenced the risk of bulbar SALS onset (genotype distribution in the cases, n=43: CC −7, 16.3%, CT −19, 44.2%, TT −17, 39.5%, genotype distribution in the controls, n=404: CC −106, 26.2%, CT −193, 47.8%, TT −105, 26.0%, p=0.05) (allele distribution in cases: C −33, 38.4%, T −53, 61.6% vs. controls: C −405, 50.1%; T −403, 49.9%, p=0.038).

Conclusion: SERPINA3 signal region A/T polymorphism is a risk factor of bulbar onset of SALS in a Polish population.

P150 THE PREDICTED LEVEL OF SMN PROTEIN IS ASSOCIATED WITH THE PHENOTYPE OF SPORADIC LOWER MOTOR NEURON DISEASE

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Background: ALS can be considered to be part of a spectrum of other motor neuron disorders characterized by predominant lower motor neuron signs to predominant upper motor neuron signs. At both ends of this spectrum are adult onset lower motor neuron disease (LMND) and primary lateral sclerosis (PLS). Furthermore, the distinction between multifocal motor neuropathy (MMN), a treatable disorder, and LMND can be difficult.

The survival of motor neuron gene (SMN), the causative gene for childhood-onset spinal muscular atrophy (SMA), was previously shown to be associated with the risk of developing LMND and ALS. In addition, copy number variation of SMN that predicts a lower smn protein expression, was shown to be associated with the severity of ALS.

Objectives: To show that copy number variation of SMN was associated with more extensive involvement of body regions in LMND, and its rate of progression.

Methods: We determined SMN1 and SMN2 copy numbers with a quantitative competitive PCR method in 47 patients with generalized LMND, 42 with non-generalized LMND, and 175 healthy controls. We used a formula taking into account that SMN2 yields only 30% of full-length SMN transcripts (estimated total full-length smn protein level=SMN1 copy number+0.3 × SMN2 copy number) to estimate total smn protein levels. In addition, we determined absence of SMN2 in 34 MMN patients.

Results: We observed an over-representation of one copy of SMN1, representing SMA carrier status, in the generalized LMND group (OR=5.3, 95% CI 1.2–24.7). In contrast, SMA carriers were not found in the non-generalized group. SMN2 copy numbers appeared to be lower in both groups, especially in the generalized group. A clear association was found between predicted level of smn protein in the generalized LMND group ($p=0.005$) but not in the non-generalized LMND group ($p=0.10$). Although numbers were small, predicted level of smn protein was not different between rapidly progressive and slowly progressive generalized LMND ($p=0.50$). A homozygous deletion of SMN2 was present in one MMN patient (2.9%; $p=0.32$).

Conclusion: This study showed that phenotypic variation in MND can be partly explained by genetic variation. SMN haplotypes that predict a lower level of smn protein were associated with generalized LMND, in contrast to a lack of association with focal variants of LMND. In addition, a homozygous deletion of SMN2 was not associated with MMN in contrast to a smaller previous study.

P151 EVALUATION OF THE ROLE OF EXOGENOUS RISK FACTORS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: The incidence of amyotrophic lateral sclerosis (ALS) in Wakayama Prefecture is higher than that elsewhere in the world. Recently, however, this incidence has gradually decreased, particularly in men, and the age at onset has shifted to the elderly, indicating the possible role of exogenous factors in the development of ALS.

Objectives: The purpose of this study is to evaluate exogenous factors in ALS development.

Methods: Data of all patients (over 40 years old) consecutively admitted to our hospital between 1999 and 2004 were collected. The ALS patients were diagnosed using the El Escorial criteria. The associations of cervical spondylosis or spinal spondylotic myelopathy (CS/SSM), surgical treatment for CS/SSM, history of bone fracture, occupations at disease onset and cervical MRI findings were compared between definite ALS patients and neurological controls. Cervical MRI findings were classified into four grades (G0 to G3) according to the severity of spinal cord compression.

Results: We analysed the data of 108 definite ALS patients (male: 62%, female: 38%; age at onset 67 ± 11.1, mean $\pm$ SD years) and 302 neurological controls (male: 52.8%, female: 47.2%; age at onset 63.1 ± 10.4 years). The frequencies of having CS/SSM, surgical therapy and bone fracture in the ALS patients were significantly higher than those in the controls (ORs: 3.7, 4.3, and 2.1, respectively). The percentage of ALS patients with G3 cervical MRI findings was significantly higher than that of the controls (OR: 4.7). The percentage of ALS patients who had secondary industrial occupations at disease onset was higher than that of the controls (OR: 2.8).

Conclusions: Continuous spinal cord compression or minor injury might be a risk factor in developing/triggering or worsening ALS.

P152 LIFESTYLE FACTORS AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS: A CASE-CONTROL STUDY IN JAPAN

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Background: Few studies have examined the relationship between lifestyle factors and the risk of ALS.

Objective: We examined associations between lifestyle factors and the risk of ALS using a population-based case-control study from 2003 to 2004 in Aichi Prefecture.

Methods: The study comprised 153 ALS patients diagnosed by El Escorial World Federation of Neurology criteria, and 306 gender- and age- matched controls randomly selected from the general population. Information on lifestyle was collected using a self-administered questionnaire. The strength of association between ALS and a potential risk factor was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Heavy exercise, self reported stress, type A behavior pattern, and less frequent intake of green-yellow vegetables were significantly associated with increased risk of ALS. Smoking and drinking habits were not associated with increased risk of ALS. The greatest effect on risk for

ALS was the combination of type A behavior pattern and less frequent intake of green-yellow vegetables.

Conclusion: These data suggested that imbalance between excessive production of endogenous oxidative stress and the decrease or lack of antioxidant defence in brain nerves may increase the risk of ALS.

P153 CASE-CONTROL STUDY FOR ALS RISK FACTORS IN A RUSSIAN POPULATION

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Background: ALS is a fatal neurodegenerative disorder with an aetiology linked with a combination of genetic and environmental factors. Significant risk factors, according to foreign authors, are male gender, age over 50 years, cigarette smoking, rural living, mechanical injury, sports and physical labour. Possible factors include contact with heavy metals and some others. Little is known about plausible mechanisms and reproducibility of these factors in different populations. We conducted the first case-control study of ALS environmental risk factors in the Russian population.

Materials and methods: We asked 100 definite ALS patients from Moscow ($n=59$), Yaroslavl ($n=27$) and Smolensk regions ($n=14$) with diagnosis verified by EMG and MRI, as well as 100 inpatients from Moscow City Hospitals 20 and 31 who have no ALS and were matched by age and gender to ALS patients, to complete the questionnaire of 17 items relating to exposures in the six months preceding disease onset. We measured Odds ratios and performed χ^2 tests to calculate frequencies.

Results: The following factors were encountered in the control group with significantly prevailing frequency: 1) surgery (OR 0.053, $p=0.0001$), 2) vegetarianism (OR 0, $p=0.09$), 3) contact with organic solvents (OR 0.26, $p=0.016$), 4) contact with magnetic fields (OR 0.22, $p=0.028$), 5) tick bites (OR 0.59, $p=0.039$), 6) acute infectious disease (OR 0.29, $p=0.04$), 7) physical labour (OR 0.2, $p=0.016$), 8) sports (OR 0.39, $p=0.0001$) and tumours in consanguine relatives (OR 0.5, $p=0.067$). Only the use of more than 200 g of alcohol per week significantly prevailed in the ALS group (OR 2.68, $p=0.027$). Cigarette smoking and contact with heavy metals in the ALS group had high OR (1.57 and 2.17), but low p by χ^2 test (0.19 and 0.44, respectively).

Conclusions: These results are in partial conflict with results obtained in previous Western studies; risk factors such as physical activity and sports dominated in Western ALS groups and alcohol abuse was not a significant risk factor for ALS in Western studies. This controversy shows that such risk factors are dependent on different lifestyle habits in Western and Russian populations, and probably on some universal factors common to ALS patients such as high intellectual and professional facilities, that are differently estimated in different societies. However, controversies were revealed also for factors independent of life habits, such as mechanical injury, contacts with magnetic fields and organic solvents. At the same time, our findings confirm the absence of association with ALS for such factors as tick bites, acute infectious disease and vegetarianism, as well as for the presence of an association between ALS and cigarette smoking and contacts with heavy metals. Thus, it is feasible not only to verify the data obtained in a larger sample but also to perform a combined analysis of genetic and environmental risk factors of ALS in separate populations.

P154 EPIDEMIOLOGY OF FALLS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: ANALYSIS OF CROSS-SECTIONAL AND PROSPECTIVE COHORTS IN THE ALS CARE REGISTRY

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Background: Falls in selected populations are dependent on the frequency of assessment. Annual occurrence of at least one fall increases with age from 28–35% ($\geqslant$65 years) to 32–42% ($\geqslant$75 years) in community dwellers. Institution dwellers have $>50\%$ chance of a single fall in the next year and previous fallers have a 67% chance of a second fall in the subsequent year (1). Annual occurrence of at least one fall is 62% due to postural instability in Parkinson's disease, 57% due to cardiovascular syncope in patients with syncope, 48% due to weakness in polyneuropathy patients and 12% due to seizures in epilepsy patients (2).

Objectives: To determine monthly occurrence of falls per ALS patient in ALS CARE Registry cross-sectional and prospectively studied longitudinal cohorts.

Methods: Analysis of ALS patients entered in ALS CARE Registry during the decade 1996–2005.

Results: Falls occurred prior to enrollment in 32% (1548/4817) of evaluable ALS patients. These patients had a median age of 61 years, median time from symptom onset of 2.4 years and median time from diagnosis of 1.5 years. Site of onset in evaluable ALS patients (413) with falls was 47% lumbar, 27% cervical, 21% bulbar and 5% thoracic. Riluzole use was 50% in ALS patients with falls as in the overall ALS CARE registry.

A prospective longitudinal cohort of ALS patients demonstrated 32% (90/280) falls at six months follow-up (5.3 falls/month per 100 patients), 27% (76/278) falls at 12 months follow-up (4.5 falls/month per 100 patients) and 29% (37/129) falls at 18 months follow-up (4.8 falls/month per 100 patients). These follow-up intervals correspond to a median time post-diagnosis of 0.8, 1.2 and 2.0 years, respectively. The annualized occurrence of falls in these ALS patients was 64%, 54% and 58%, respectively. Riluzole use in ALS patients with falls was 52% at six months follow-up, 59% at 12 months follow-up and 68% at 18 months follow-up, while median age was 61, 59 and 60 years, respectively.

Discussion and conclusions: The rate of falls per month per 100 ALS patients in a cohort followed prospectively appears to be relatively stable (5.3 (0.8 years post diagnosis), 4.5 (1.2 years post diagnosis) and 4.8 (2.0 years post diagnosis)). Further analysis is required to determine if this rate is stable because there is a balance between the later development of falls in ALS patients who will have lower fall rates and the removal of ALS patients with increasing fall rates or the marked reduction in fall rates in ALS patients with increasing fall rates through the introduction of adaptive devices and increased emphasis on attention to patient safety.

The observed rate of falls in ALS patients provides a benchmark for future clinical studies and safety analyses for clinical trials. The observed rate is slightly higher than the rate for polyneuropathy patients and similar to that for Parkinson's disease patients. The role of site of ALS onset, time to develop leg weakness and time to develop impaired postural reflexes in the pathophysiology of falls requires further study.

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P155 ETHNIC VARIATION IN THE INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW

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Background: The findings of recent genetic polymorphism studies in amyotrophic lateral sclerosis suggest that the influence of genetic risk factors for the disease may vary by ethnicity. It is now widely accepted that the incidence of ALS is uniform across Caucasian populations, but whether racial variation across other ethnicities exists remains unknown.

Methods: We performed a systematic review of the known literature on the incidence, prevalence and mortality of ALS across all ethnicities, paying particular attention to sources of case finding, inclusion criteria and to studies which stratified their findings by ethnicity.

Results: The literature search identified 27 European incidence studies and 17 conducted in other ethnicities. Twenty-two of these provided sufficient data for age- and sex-adjustment to a standard population allowing direct comparison.

To date, the only well-conducted prospective incidence study which has directly compared the frequency of ALS in those of black African descent with a Caucasian population was underpowered to report the lower incidence with certainty. Within the United States, a number of other retrospective incidence studies and mortality studies consistently support the notion of an African resistance to ALS. There are no reliable incidence or mortality studies from the African continent itself, while migration studies conducted in London and Israel provide restricted evidence of lower disease frequency in Africans.

Population-based epidemiological studies from Asia provide evidence of a lower age- and sex-adjusted incidence of ALS when directly compared to the adjusted incidence in Caucasian populations. However, a similar effect was not seen in an ethnically stratified incidence study from Hawaii.

Numerous epidemiological studies from Central and South America provide crude incidence and mortality rates for ALS in Hispanic populations that are well below non-Hispanic Caucasian averages. None of these can be adjusted to a standard population for direct comparison. However, when compared to non-Hispanic white subjects in the United States, lower female incidence and overall crude mortality among Hispanics has been described.

Discussion: Our findings from the limited available data, although imperfect, suggest that the incidence of ALS may be lower among African, Asian and Hispanic ethnicities than among Caucasians. We conclude that a well-designed prospective epidemiological study concentrating on non-Caucasian populations should be prioritized.

P156 REAL TIME STATE-LEVEL PUBLIC HEALTH SURVEILLANCE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND MULTIPLE SCLEROSIS (MS): PRACTICAL GEOGRAPHIC IMPLICATIONS OF WISCONSIN'S ALS MORTALITY EXPERIENCE

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Background: As electronic data systems for health outcomes and environmental contaminants have advanced, it has become increasingly apparent that governmental health and environmental data are largely not collected or aggregated in a way that supports public health surveillance efforts for chronic conditions for which environmental factors are likely contributors.

Objective: Wisconsin's Environmental Public Health Tracking (EPHT) program has undertaken a demonstration project to enhance surveillance efforts for

amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) and create a platform for generating and testing environmental hypotheses about ALS etiology by linking case information with statewide environmental hazard data. For geographical linkage, accurate geographical localization of ALS patients will be necessary.

Methods: To this end, mortality data from Wisconsin's vital records system for 1989 through 1998 were extracted and analysed in order to summarize how deaths were classified and to determine the degree to which geographically-specific estimates of environmental hazards could be considered representative of decedent exposure.

Results: For the 1989–1998 period, a total of 1047 deaths were identified in Wisconsin for which ALS was derived as underlying cause of death (UCOD) or listed as a primary cause of death. ALS was listed as the UCOD in 508 deaths (48.5%). In cases where ALS was not specified as the UCOD, respiratory arrest, pneumonia and death due to inhalation of food or vomitus were most commonly reported as the UCOD. For the 1989–1998 period, ALS deaths increased from 72 per annum to 116 per annum. Among the 658 deaths reported as occurring outside an inpatient facility, 280 (42.6%) were classified as occurring in nursing homes rather than the decedent's place of residence.

Discussion and conclusions: From these observations, it is clear that residential history information beyond what is available from state vital records is sorely required to adequately consider environmental hypotheses about the incidence of ALS. As such, successful investigations of the role of environmental hazards in ALS incidence would be greatly aided by patient- and decedent-level information about residential history, and adds to the impetus to develop national and international ALS registries for real time localization of incident ALS patients for proper environmental association studies.

P157 INTERNAL AUDIT OF ALS CARE REGISTRY: BASELINE DEMOGRAPHIC CHARACTERISTICS THAT MAY DISTINGUISH ALS PATIENTS WITH AND WITHOUT FOLLOW-UP DATA

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Background: The 'ALS CARE Registry' (ALSCR) is currently the largest clinical outcomes database with ALS clinic-based or neurologist-based verification of the diagnosis of ALS. Data are available for 5171 enrolled patients. Follow-up data are available: 2162/5171 ALS patients (41.8%) have died with death verified by Completion Form; 2430/5171 patients (47.0%) are known to be alive overall; 579/5171 patients (11.2%) are truly lost to follow-up. However, only 1226/5171 patients (23.7%) are alive with follow-up observations while 163/5171 patients (3.2%) left the study at enrollment as alive and 1041/5171 patients (20.1%) were enrolled as alive and have no follow-up or verification by Completion Form. Two groups are defined as the 'Survival Cohort' (SC) of 2760/5171 ALS patients with follow-up data of various intervals (53.4%) and the 'Non-Survival Cohort' (NSC) of 2411/5171 ALS patients without follow-up data (46.6%).

Objective: To compare the baseline characteristics of the SC with the baseline characteristics of the ALSCR and of the NSC to determine whether there are distinct systematic differences which might define follow-up experience.

Methods: Group differences and distributions were compared with descriptive statistics.

Results: Male: female ratio (59% (M):41% (F)) was identical in SC and ALSCR. Other comparisons include White/Caucasian patients (93% (SC); 92% (ALSCR)), education level college and higher (42%; 38%), annual income level $60,000 or higher (19%; 19%); married (78%; 76%); spinal surgery (5%; 5%); sporadic ALS (94%; 94%); bulbar onset (30%; 30%). In the SC there was no significant baseline difference in educational level, or income level between those who died and those who did not. There was a significant difference at baseline in the M:F ratio (56%: 44% vs. 62%: 38% ($p=0.0026$)); Oriental/Asian, Pacific Islander-North American Indian, Eskimo-Black/African American patient (3.6% vs. 6.2% ($p=0.006$)); never married-divorced-separated patient (14% vs. 19% ($p=0.014$)) characteristics of the SC, but no significant difference in the type of ALS, site of initial disease or experience of antecedent spinal or peripheral nerve surgery. Baseline characteristics that may determine poor follow-up identified in the NSC include North American Indian, Eskimo-Black/African American patients (5.5% vs. 7.2% ($p=0.0077$)); income below $40,000 (45% vs. 51% ($p<0.0001$)); never married-divorced-separated-widowed (22% vs. 26% ($p=0.0045$)) but no significant difference in M: F ratio, education level or riluzole use at baseline.

Conclusions: In the ALSCR the SC differs from the NSC in specific baseline demographic parameters which may explain the difference in follow-up. These baseline demographic characteristics were different from those characteristics present in the SC ALS patients who were known to survive and those who were known not to survive.

P158 A TELEPHONE SCREENING METHOD TO IDENTIFY ALS CASES IN A POPULATION: VALIDATION AGAINST MEDICAL RECORD REVIEW IN THE VETERANS AFFAIRS ALS REGISTRY

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Background: A major goal of the National Registry of Veterans with ALS is to identify US military veterans with ALS and ascertain their clinical status. We identify potential ALS cases from several sources using both active and passive strategies, including surveying VA databases, patient self-referrals to a web site, and toll-free phone number. In order to limit the false positive rate of ALS diagnosis among Registry candidates, we devised a telephone screening method to eliminate subjects with erroneous diagnoses prior to medical record validation. We now report the effectiveness of this approach.

Objectives: The aim of this study was to ascertain the rate of false positive diagnosis of ALS using the telephone screening method compared to an established diagnosis by medical record review by a neurologist with expertise in ALS diagnosis.

Methods: A total of 3161 potential ALS registrants were identified and screened from all sources. Each was contacted by a trained interviewer. After telephone informed consent, each candidate was screened using two primary questions: 1) Did any health professional diagnose you with ALS or other MND, and 2) Have you experienced progressive muscle weakness? Other demographic variables were also obtained. Of those screened, 1356 (42.9%) were excluded. The primary reason for exclusion was not having a diagnosis of ALS or progressive muscle weakness (74.0%). Other participants were excluded for other reasons, refused participation, died prior to record review, or did not yet have enough medical evidence to determine a diagnosis. One thousand, five hundred and twelve participants proceeded to medical record review. Levels of diagnostic certainty were established using the El Escorial criteria.

Results: The population was predominantly male (97.8%) and Caucasian (93.0%) with a mean age of 63.8 ± 11.2 years. Considering participants identified from all sources, 90.6% who passed the telephone screener proved to have ALS/MND on medical record review and 9.4% did not have ALS/MND. Seven hunred and eighty-four subjects were self-referred and of these, 96.4% had ALS/MND on record review and 3.6% did not, whereas of the 728 potential registrants obtained from databases, 15.6% did not have ALS/MND upon record review.

Conclusions: The results of this study indicate that a telephone-based screening method using questions targeting the presence of progressive muscle weakness and a suggested diagnosis of ALS/MND by a health profession is associated with a low false positive rate of diagnosis using medical record review by experts as the 'gold standard'. This was especially true when the source was self-referral, implying that such candidates received an accurate diagnosis, and confirmed by the history of progressive weakness over the phone. Not unexpectedly, computerized data sources carried a higher false positive rate but we did not establish the source of error in this study. Our results suggest a strategy with a low false positive rate of ALS that can be used to screen large numbers of potential study candidates for population-based research in a cost-efficient manner.

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P159 DECREASING INCIDENCE AND RISING PREVALENCE OF ALS IN IRELAND OVER A SEVEN-YEAR PERIOD

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Introduction: There has been a longstanding debate concerning the temporal trend of ALS incidence. However, there has been a lack of reliable, population-based data concerning changes in ALS epidemiology over the last decade since disease-modifying agents were introduced and access to symptomatic therapies that influence patient prognosis became more available.

Aim: The authors sought to determine the incidence and prevalence of ALS in Ireland from the three-year period 2002 to 2004 and to compare it to the previously published findings from the three-year period 1995 to 1997.

Design: Population-based register of all individuals diagnosed with ALS in Ireland using multiple sources of information to ensure complete case ascertainment. To compensate for the recent shift in the demographics of the Irish population, the 2002– 2004 figures were standardized to the 1996 Irish census.

Results: Two hundred and thirty-five residents of Ireland were diagnosed with ALS during the three-year study period between 1 January 2002 and 31 December 2004. The average annual incidence of ALS for the time period 2002–2004 was 2.50 per 100,000 person-years over the age of 15 years (95% CI 1.91–3.09). This represents a 10.1% decrease compared to the incidence of ALS for the

1995–1997 time period (2.78 per 100,000 person-years over the age of 15; 95% CI 2.42–3.44), but this difference did not reach statistical significance. The drop in incidence was distributed equally among men and women: the average annual incidence among men for 2002–2004 was 10.7% lower than in 1995–1997 (2.91 per 100,000 (95% CI 2.00–3.82) compared to 3.26 (95% CI 2.71–3.81)). The average annual incidence among women was 10.3% lower (2.08 per 100,000 (95% CI 1.33–2.84) compared to 2.32 (95% CI 1.88–2.83)). In contrast to incidence, the overall prevalence of ALS in Ireland grew by 2% between 1995 and 1997 and 2002–2004: on 31 December 2003, ALS prevalence was 6.34 per 100,000 population over the age of 15 years (95% CI 5.40–7.28), whereas on 31 December 1996 prevalence was 6.20 per 100,000 (95% CI 5.3–7.1). The majority of this increase in prevalent cases was accounted for by women, in whom ALS prevalence increased by over 10% (4.99 per 100,000 in 2003 compared to 4.50 per 100,000 in 1996). In contrast, male prevalence diminished by 2.4% (7.71 per 100,000 in 2003 (95% CI 6.24–9.19) compared to 7.9 (95% CI 6.4 –9.4) in 1996.

Conclusions: The incidence rate of ALS in Ireland has decreased over the seven-year period from 1995–1997 to 2002 –2004, whereas the prevalence rate has increased over the same time period. This temporal pattern may reflect changing practices in the diagnosis of ALS (e.g. exclusion of 'suspected' ALS cases). Alternatively, the advent of riluzole and the adoption of a more aggressive approach to symptomatic management may be improving survival among Irish ALS patients and may explain the rising prevalence of this fatal neurodegenerative disease in Ireland.

P160 MORTALITY AND SURVIVAL OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS IN BELGRADE, SERBIA

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Background: Over the last decade increased mortality of ALS has been reported especially in European countries (1). In our first epidemiological study of ALS in Belgrade (Yugoslavia) the adjusted mortality rate was low: 0.29 per 100,000 population in a seven-year period (1985–1991) (2). The cumulative probability of five-year survival was 27%.

Objectives: To determine the mortality of ALS in the population of Belgrade (Serbia) during the period 1993–2005 and to estimate five-year survival rates.

Methods: ALS cases were collected by analysing hospital inpatient and outpatient registers at the Institute of Neurology which is the national referral neurological centre and in departments of neurology in an additional three clinical centres in Belgrade. The El Escorial diagnostic criteria for ALS were applied to all cases enrolled in the register. Each patient was regularly followed up during the disease.
The mortality rates were calculated by standard procedures. The probability of survival was calculated by the Kaplan-Meier method.

Results: During the period l993 to 2005, 218 patients were diagnosed as new cases of ALS in the District of Belgrade. Of these, nine (3.7%) had familial ALS based on detailed family history. Altogether, 175 patients (102 males and 73 females) died before 31 December 2005. The youngest patient (male) was 24 years old and the oldest patient (male) was 85 years old. Twenty-six (11.9%) patients were under the age of 45 years. The mean age of onset was 58.5 ± 11.4 (range 24–85) years.
The overall mortality rate was 0.8/100,000: 1.0/100,000 for males, and 0.7/100,000 for females. During the observed period the mortality rate of ALS in Belgrade showed an increasing trend ($y=0.503+0.050x$, $p=0.103$). The mean survival time from the onset of disease was 3.4 ± 2.3 years and cumulative probability of five-year survival was $22.8 \pm 3.1\%$. Patients under 58 years at onset ($p=0.001$) and those with the spinal onset of disease survived significantly longer ($p=0.014$).

Conclusions: In comparison to our previous data the results suggest a significant increase of mortality in Belgrade (Serbia). The most important contributing factors are better diagnosis due to El Escorial criteria, more thorough registration of ALS and increased life expectancy. However, a real increase in the mortality of ALS, possibly related to environmental factors, cannot be excluded.

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THEME 9 HUMAN CELL BIOLOGY AND PATHOLOGY

P161 PALLIDO-LUYSO-RUBRO-NIGRAL ATROPHY ASSOCIATED WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS: A CLINICAL, GENETIC AND PATHOLOGICAL STUDY

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Background: Atypical manifestations in ALS include sensory impairment, dementia, ocular palsy, bladder/bowel dysfunction, decubiti, cerebellar and extrapyramidal signs. These special forms of ALS are defined as ALS Plus. Hyperkinetic movements in ALS are very rare.

Objectives: To report the clinical, genetic, and neuropathological findings in one woman with clinical criteria of familial ALS who, 24 months after onset of motor symptoms, presented hyperkinetic movements consistent with choreoathetosis and ballism.

Methods: A 53-year-old woman suffered from progressive weakness and amyotrophy in the lower and then upper extremities, breathing difficulties, neurogenic bladder and speech and swallowing difficulties consistent with ALS. A similar disease had occurred in her father and her older sister. Two years after initial motor symptoms, choreic movements affecting the face, mouth, neck and hands appeared, together with continuous, rotary excursions of the upper limbs at proximal joint level, mimicking ballism. The patient died of respiratory failure 27 months after initial motor deficit. Genetic studies for *IT15, DRPLA,* and *SOD1* gene mutations were performed. Brain and spinal cord were obtained six hours after death and subsequent neuropathological examination was performed.

Results: HD, DRPLA, ALS1, acanthocytosis, Wilson's disease, thyroid and parathyroid disorders were ruled out by appropriate genetic studies, peripheral blood smear examination, ceruloplasmin, TSH and PTH levels.

Neuropathological examination showed neuron loss in the anterior horn of the spinal cord and motor nuclei of the medulla oblongata, and atrophy and myelin pallor of the anterior spinal roots and pyramidal tracts, as well as of the spinocerebellar, rubrospinal and vestibulospinal tracts with preservation of the posterior columns. In addition, neuron loss and gliosis occurred in the internal globus pallidus, subthalamus, substantia nigra pars compacta and red nucleus. Intranuclear inclusions were absent.

Conclusions: The main clinical features in this patient with ALS Plus were the presence of hyperkinetic movements 24 months after the onset of leg weakness. Neuropathological examination showed involvement of the spinocerebellar, rubrospinal, reticulospinal and vestibulospinal tracts, and upper and lower motor degeneration. Most important in the present context was degeneration of the internal globus pallidus, subthalamic nucleus, substantia nigra and red nucleus. This combination together with FALS is unique, although it probably should be considered within the spectrum of ALS Plus with pallido-luyso-nigral atrophy. These data support the concept that extrapyramidal symptoms in ALS Plus may be the result of associated pallido-luyso-(rubro-)nigral atrophy. Considering that this association is not unusual in ALS Plus cases, pallido-luyso-rubro-nigral atrophy can be considered as a particular manifestation in certain sporadic and familial ALS cases. The cause of this multisystemic atrophy is not related to HD, DRPLA or SOD1 gene mutations.

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P162 RNA EDITING IN SPORADIC ALS AND OTHER MOTOR NEURON DISEASES

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Background: Deficient RNA editing of GluR2 mRNA at the Q/R site has been proposed to be the cause of death in spinal motor neurons in sporadic ALS. RNA editing at the GluR2 Q/R site is specifically catalysed by adenosine deaminase acting on RNA type 2 (ADAR2). Therefore, underediting of GluR2 in ALS motor neurons is likely to be due to a decrease of the ADAR2 enzyme activity. The aim of this study is to investigate whether the reduction of RNA editing of the GluR2 Q/R site occurs in motor neuron diseases other than sporadic ALS, and whether the ADAR2 activity is reduced in ALS motor neurons compared with control motor neurons.

Methods: With a laser microdissector, motor neurons were dissected from frozen autopsied spinal cord of spinal and bulbar muscular atrophy (SBMA) patients and symptomatic rats transgenic for mutated human Cu/Zn superoxide dismutase (SOD1) (G93A, H46R). In addition, ventral grey matter tissue was dissected en bloc under

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a microscope from frozen autopsied human control and ALS spinal cords. Dissected tissues were collected and were subjected to RT-PCR. The editing efficiency of ADAR2 substrates including the GluR2 Q/R site was determined by the quantitative analysis of the fragments of the PCR products digested by a restriction enzyme, and the expression levels of ADAR2 mRNA and GluR2 mRNA were measured using LightCycler system.

Results: We found that GluR2 mRNA was completely edited in all the motor neurons of SBMA patients and SOD1 transgenic rats examined. The expression level of ADAR2 mRNA was significantly decreased only in the ventral grey matter of the ALS spinal cord. Among the various known ADAR2-catalysed editing sites we examined, RNA editing was significantly reduced only at the GluR2 Q/R site in ALS ventral grey matter compared with the control ventral grey matter.

Conclusions: Motor neurons in mutated SOD1 transgenic rats and SBMA patients may die via mechanisms other than deficient RNA editing, hence there seem to be multiple death pathways in motor neurons. Furthermore, underediting of the GluR2 Q/R site is the molecular mechanism unique to death of motor neurons in sporadic ALS. A reduction of the ADAR2 activity is likely to be the cause of deficient GluR2 RNA editing. Although the expression level of ADAR2 mRNA is one of the regulatory factors of the ADAR2 activity *in vivo*, it is likely that there are other factors regulating the ADAR2 activity at the Q/R site of GluR2 mRNA.

P163 ALSIN, A CAUSAL GENE PRODUCT OF AMYOTROPHIC LATERAL SCLEROSIS 2, PHYSICALLY INTERACTS WITH TOLLIP AND REGULATES NF-κB FUNCTION

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Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disease. The mutated *ALS2* gene has been identified as a cause of the recessive inherited juvenile ALS (ALS2). The *ALS2* gene encodes a large protein termed alsin, which contains a number of predicted sequence motifs of cell signalling and protein trafficking. Alsin is expressed in various tissues and cells, including neurons of brain and spinal cord, but the molecular mechanism of the ALS2-related motor neuronal death remains unclear.

Objectives: To gain insight into the mechanisms of neuronal cell death in ALS2, we searched for the alsin-interacting protein by using the yeast two-hybrid system. We hypothesized that the downstream signaling pathway of alsin and its interacting molecules may play a key role in regulating motor neuron death in ALS2.

Methods: pBluescript II SK (+) KIAA1563 contains a part of ALS2. We used human brain cDNA library as a template to create the remainder constructs using amplification by polymerase chain reaction (PCR) to obtain the full length of ALS2 cDNA. We used the yeast two-hybrid system to screen for proteins interacting with alsin. We constructed the plasmids of each domain of alsin. The physical interaction of alsin with the proteins identified by the yeast two-hybrid system was confirmed by using immunoprecipitation. The transfection method was used for overexpression of alsin and other proteins in cells. Cell death was measured by Trypan blue dye exclusion assay and by fluorescence activated cell sorter (FACS) analysis. For transcriptional activation assay, we used luciferase reporter assay.

Results: The yeast two-hybrid system identified Tollip (Toll-interacting protein) as a binding partner of alsin. By immunoprecipitation assay, Tollip was found to specifically bind to the MORN motifs domain of alsin. Overexpression of Tollip induced cell death in HEK293 and NSC34 cells. Interestingly, the cell death was inhibited after co-transfection of ALS2 and Tollip in those cells. Furthermore, as a Toll/IL-1R domain-containing adaptor protein, Tollip formed a complex with IRAKs (IL-1RI-associated protein kinases) family, thereby preventing IRAK-1-induced-NFκB activation. In our studies, Tollip inhibited TNFα-induced NFκB activation as in IL-1R/TLR signalling. The decreased NFκB activity was recovered after co-transfection of ALS2 and Tollip.

Discussion and conclusions: In the present study, we first found that Tollip is a specific binding protein with alsin at its MORN motifs domain. Our present results suggest that alsin interacts with Tollip to inhibit its suppression of NF-κB activation. The mutated alsin might not be able to inhibit Tollip, thereby neuronal cell death could be induced.

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P164 COLLAGEN ABNORMALITIES IN PYRAMIDAL TRACT AND ANTERIOR HORN OF THE SPINAL CORD IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Studies of skin in patients with amyotrophic lateral sclerosis (ALS) have reported morphological and biochemical abnormalities of collagen. These findings, which were not found in controls, could be one of the characteristics of ALS. However, little is known concerning collagen of the spinal cord in ALS.

Objectives: To clarify collagen alterations in the spinal cord of ALS.

Methods: We measured the amount of collagen and characterized collagen at an electron microscopic level in the posterior funiculus, posterior half of the lateral funiculus and anterior horn of cervical enlargement of the spinal cord obtained from 15 patients with ALS, 15 patients with other neurological diseases (control group A), and 15 patients without neurological disease (control group B).

Results: In the posterior half of the lateral funiculus and anterior horn: 1) ultrastructually, collagen bundles were more fragmented and widely separated, and the fibrils were randomly orientated in the perivascular space of capillaries in ALS patients, which were not observed in any areas of control groups or in the posterior funiculus of ALS patients; and 2) the collagen contents in ALS were significantly lower than those in control groups A and B ($p < 0.001$ and $p < 0.001$, respectively).

Discussion and conclusions: These morphological changes of collagen in the interstitial tissue surrounding capillaries, and markedly decreased amount of collagen in the posterior half of the lateral funiculus and in the anterior horn in ALS, could be related to the degeneration of the upper and lower motor neurons in the spinal cord, i.e. selective neuronal involvement in ALS.

P165 INCREASED EXPRESSION OF NEUROTROPHIC FACTORS IN THE SKIN OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Our previous reports have shown unique morphological and biochemical abnormalities in the skin of patients with amyotrophic lateral sclerosis (ALS). One of the features of patients with ALS is the absence of bedsores. Neurotrophic factors including CNTF, IGF-I, and LIF have been found to affect the survival and maintenance of a variety of neuronal cell types, including motor neurons. In addition, neurotrophic factors have been implicated in the pathogenesis of various neurodegenerative disorders such as ALS and evaluated as a therapeutic agent for the treatment of ALS. Little is known, however, about whether the expression of neurotrophic factors is altered in skin of ALS patients.

Objectives: To study the expression of ciliary neurotrophic factor (CNTF), insulin-like growth factor I (IGF-I), and leukaemia inhibitory factor (LIF) in skin from ALS patients, compared with controls.

Methods: Skin biopsy samples were taken from the left upper arm of 15 patients with ALS (11 men and 4 women, mean age 57.3 years) and from 15 controls with other neurodegenerative diseases matched for sex and age (11 men and 4 women, mean age 60.4 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. The sections were incubated with anti-CNTF antibody, anti-IGF-I antibody, and anti-LIF antibody. After washing in phosphate-buffered saline, biotinylated anti-IgG was applied. The sections were stained by ABC kit. The immunoreactivity was quantified with an image-analysis system. Statistical comparisons were made by the two-tailed Student's t- test with $p < 0.05$ as the significance level. Correlation coefficients were calculated by the least-squares method.

Results: The immunoreactivities of CNTF, IGF-I, and LIF were strongly positive in the epidermis and in some blood vessels and glands of the reticular dermis in all ALS patients but not in controls. These findings became more conspicuous as ALS progressed. The optical densities of CNTF, IGF-I, and LIF immunoreactivities in ALS patients were significantly higher than in controls. Furthermore, there was a significant positive relationship between the immunoreactivities of CNTF, IGF-I, and LIF and duration of illness in ALS patients. There was no relationship between the optical densities of CNTF, IGF-I, and LIF and the presence of dysphagia, weight loss, muscular atrophy, loss of active movement, and bedridden state in ALS patients or controls.

Discussion and conclusions: CNTF, IGF-I, and LIF were found to be significantly up-regulated in skin samples from ALS patients. The results suggest that changes of CNTF, IGF-I, and LIF in the skin of ALS patients are likely to be related to the disease process. These neurotrophic factors may have a trophic role in the skin of ALS patients and may help to explain why decubitus formation is rare in ALS.

P166 AN INCREASE OF OXIDIZED COENZYME Q-10 OCCURS IN THE PLASMA OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Oxidative stress has been suggested to underlie pathogenesis of amyotrophic lateral sclerosis (ALS) since missense point mutations occur in the copper/zinc (Cu/Zn) superoxide dismutase (SOD) gene of familial ALS patients. Indeed, elevated levels of thiobarbituric acid reactive substances (TBARS) and 8-hydroxy-deoxyguanosine (8-OHdG) were found in the plasma of sporadic ALS patients compared with controls. Nevertheless, plasma and serum levels of antioxidants such as vitamin E, ascorbic acid, coenzyme Q-10 and carotenoids were not different between sporadic ALS patients and healthy controls. Conflicting results of antioxidant enzyme activities such as SOD and glutathione

peroxidase were also obtained in the plasma or serum of ALS patients.

Objectives: The aim of the present study is to examine the levels of several antioxidants and the redox status of coenzyme Q-10 in the plasma of sporadic ALS patients.

Methods: Plasma samples were collected from 20 sporadic ALS patients and 20 controls. We applied a sensitive and reliable method with high performance liquid chromatography for the simultaneous detection of ubiquinol-10 ($CoQH_2$-10, the reduced form of coenzyme Q-10) and ubiquinone-10 (CoQ-10, the oxidized form of coenzyme Q-10) in plasma samples from sporadic ALS patients and age/sex-matched controls. Then we calculated the ratio of CoQ-10 to total coenzyme Q-10 (%CoQ-10) as an indicator of oxidative stress.

Results: We found no significant differences in plasma levels of uric acid, ascorbic acid, unconjugated bilirubin, vitamin E, $CoQH_2$-10, total coenzyme Q-10 and $CoQH_2$-10/total cholesterol between sporadic ALS patients and control volunteers. However, plasma levels of CoQ-10 ($p=0.0002$) and CoQ-10/total cholesterol ($p<0.0001$) were significantly higher in sporadic ALS patients than those in controls. In addition, %CoQ-10 values were significantly higher ($p<0.0001$) in sporadic ALS patients than in controls. A significant correlation was observed between %CoQ-10 values and the duration of illness ($p<0.05$)

Conclusion: We demonstrated a significant increase of plasma %CoQ-10 in sporadic ALS patients, suggesting systemic oxidative stress in the pathogenesis of the disease.

P167 DECREASED LEVELS OF REACTIVE OXYGEN SPECIES, SOD1 EXPRESSION AND ACTIVITY IN PERIPHERAL TISSUES OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Pathogenesis of neuronal degeneration in both sporadic and SOD1- mutated familial amyotrophic lateral sclerosis (ALS) may involve oxidative stress (1). In a previous paper (2) we demonstrated that lymphocytes from sporadic ALS patients (SALS) are more prone to undergo alteration of cell membrane integrity in both basal condition and following additional oxidative stress. Moreover, SALS lymphocytes showed reduced intracellular SOD1 levels compared to controls. Such results suggest that oxidative stress protection pathways are deregulated in sALS patients.

Objectives: The aim of this work was to assay over time SOD1 levels and activity in SALS lymphocytes and plasma samples. Moreover, plasma oxidative status was evaluated by assaying reactive oxygen species (ROS) levels.

Methods: Intracellular SOD1 expression was evaluated in lymphocytes from 10 SALS patients at the time of diagnosis and after six months. Lymphocytes obtained by Ficoll-Hystopaque 1077 gradient were processed for protein extraction and SOD1 expression was evaluated by Western blotting. Total blood SOD1 activity and plasma ROS levels were assayed in 79 SALS patients: blood samples from all patients were drawn every two months over six years. SOD1 activity (587 blood samples) was assayed at 30°C by its ability to inhibit superoxide radical-dependent reactions (3) and expressed as U/g of haemoglobin. ROS levels (468 samples) were evaluated by a colorimetric method (Diacron International, Grosseto, Italy) and expressed as U CARR.

Results: Western blotting experiments showed that intracellular SOD1 expression was significantly ($p<0.01$, Student's *t*- test) decreased after six months from the first withdrawal. Mean value of SOD1 activity evaluated for each patient was decreased in 71% of cases. Moreover, mean value of plasma ROS levels calculated for each patient was under the normal range in 61% of cases.

Discussion: Our data show that total blood SOD1 activity, reflecting both the activity of extracellular SOD and that of intracellular SOD1 released by damaged cells, is lower compared to normal values. Moreover, SOD1 expression, previously demonstrated to be significantly lower in SALS lymphocytes compared to those from controls, decreases over time in SALS patients. Taken together, these findings reinforce the hypothesis that the wild-type SOD1 protein may also be implicated in ALS pathogenesis, although the precise mechanisms are still unknown. Surprisingly, in the majority of patients circulating ROS levels were under the normal range, suggesting that ROS increase may be confined to the intracellular compartment.

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P168 A COMMON MOLECULAR SIGNATURE IN SOD1 FOR BOTH SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron degenerative disease whose etiology and pathogenesis remain poorly understood. Most cases of ALS (~90%) are sporadic (SALS), occurring in the absence of genetic associations. About 20% of familial ALS (FALS) cases are due to mutations in the copper/zinc superoxide dismutase (SOD1) gene. Molecular evidence for a common pathogenesis of SALS and FALS has remained elusive.

Objectives: The objective of this study was to identify a common molecular signature for both SALS and FALS.

Methods: We applied a new approach involving biotinylation of accessible lysine residues in proteins, followed by SDS-PAGE and Western blot (WB) analysis for SOD1. To verify that the SOD-immunoreactive (IR) protein species indeed contained the SOD1 molecule, nanospray mass spectrometry technique was used. In addition, column chromatography method was used to determine the source that gave rise to the SOD1-IR band.

Results: Biotinylation revealed a 32 kDa ALS-specific protein species immunoreactive to a peptide-specific rabbit antiserum to human SOD1. This distinctive SOD1-IR species was observed in both spinal cord and muscle of individuals with ALS, but not present in normal or non-ALS disease cases. Furthermore, we confirmed that the 32 kDa SOD1-IR contained the SOD1 molecule and was distinct and independent from normal sized SOD1 during biotinylation. Thus, covalent chemical modification allowed us to identify an attribute of SOD1 common to both SALS and SOD1-linked FALS.

Discussion and conclusions: Our studies used chemical modification as a novel tool for the detection of previously imperceptible differences and/or potential disease-associated biomarkers in the proteome. Our results suggest a shared molecular event involving a known target gene as a common step in the etiology and pathogenesis between SALS and FALS.

P169 AGGREGATION OF SOD1 IS PRIMARILY NEURONAL, RATHER THAN GLIAL IN ALS PATIENTS AND MOUSE MODELS WITH SOD1 MUTATIONS

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Background: Mutations in the Cu/Zn superoxide simutase gene (SOD1) are associated with about 20% of familial ALS cases. The pathogenic mechanisms underlying SOD1-mediated ALS are not well understood. A pathological hallmark of the mutant SOD1-mediated ALS is SOD1-immono-reactive aggregates. These aggregates are found in neurons. In a few cases, SOD1 aggregates were also reported in glial cells. The prevalence and the pathogenic role of the aggregates in glial cells are not known.

Objectives: The objective of this project is to study the cellullar distribution of the SOD1 aggregates in the spinal cords of ALS subjects, including ALS patients with SOD1 mutation and transgenic mice overexpressing ALS-associated SOD1 mutants.

Methods: The spinal cord sections from ALS patients with $SOD1^{I113T}$ and $SOD1^{G85R}$ mutations, and transgenic mice overexpressing $SOD1^{G93A}$, $SOD1^{L126Z}$ and a newly developed $SOD1^{G85R}$ mouse line (Lijun & Roos, unpublished) were analysed with immunohistochemistry and confocal microscopy using antibodies against SOD1 and GFAP. We also crossbred the $SOD1^{G93A}$ mice with EGFP (enhanced green fluorescent protein) knock-in mice at CX3CR1 locus. These mice show strong GFP in microglia.

Results: We observed SOD1-immunoreactive aggregates in large neuronal bodies and their processes. Although these aggregates were reported primarily in astrocytes in one of the $SOD1^{G85R}$ transgenic mouse lines, we were unable to identify the SOD1 aggregates in the astrocytes in our new $SOD1^{G85R}$ transgenic mice and in an ALS patient with $SOD1^{G85R}$ mutation. We did not find apparent SOD1 aggregates in glial cells in the patients with $SOD1^{I113T}$ either. We did not observe SOD1 aggregates in microglia in $SOD1^{G93A}$ mice. We observed an increased expression of SOD1 in some activated astrocytes in the transgenic mouse samples, but this SOD1 was apparently in a diffused form, not an aggregated form.

Discussion and conclusions: Thus, our data suggest that the SOD1 aggregates in glial cells may not be a common feature of the SOD1-mediated ALS. Therefore, the aggregates in neurons, rather than glial cells may play a pathogenic role in SOD1-mediated ALS.

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P170 NEDL1, A NOVEL UBIQUITIN-PROTEIN LIGASE, TARGETS MUTANT SUPEROXIDE DISMUTASE-1 AND P53 TO INDUCE APOPTOSIS

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Background: Approximately 20% of familial amyotrophic lateral sclerosis (FALS) arises from germ-line mutations in the Cu/Zn superoxide dismutase (SOD) 1 gene. Recently, ubiquitin-proteasome system impairment has been implicated in mutant SOD1-induced toxicity. However, the molecular mechanism underlying the pathogenesis of FALS remains unclear.

We have identified a novel HECT-type E3 ubiquitin ligase termed as NEDL1 (NEDD4-like ubiquitin protein ligase 1) from oligo-capping cDNA libraries generated from anonymous neuroblastoma tissues that were undergoing spontaneous repression. Northern blot analysis revealed that NEDL1 was preferentially expressed in neuronal tissues. Furthermore, NEDL1 was highly expressed in favourable neuroblastomas. These observations indicate that NEDL1 might be involved in neuronal cell death.

Objectives: In this study, we sought to investigate the role of NEDL1 in the pathogenesis of FALS as well as the molecular mechanism of induction of neuronal cell death.

Methods: A yeast two-hybrid assay was performed to identify the physiological target of NEDL1. Immunoprecipitation and immunoblotting were conducted to detect the interaction of NEDL1 with SOD1s. Immunohistochemical analysis was employed to examine the localization of NEDL1 using spinal cord sections from FALS patients and mutant SOD1 transgenic mice. Ubiquitination assay, colony formation assay, and luciferase reporter assay were performed to unveil the functional role of NEDL1 in the induction of neuronal death.

Results: Dishevelled-1, a regulatory molecule in the Wnt signaling pathway, was found as the physiological target of NEDL1 for ubiquitination and proteasome-mediated degradation. NEDL1 bound to and ubiquitinated various mutant (but not wild-type) SOD1s, in a FALS severity-dependent manner. Immunohistochemical study showed that NEDL1 aggregated with mutant SOD1s in the Lewy body-like hyaline inclusions, a typical pathological change in FALS, in the spinal cord anterior motor neurons of both FALS patients and mutant SOD1 transgenic mice. Furthermore, NEDL1 physically bound to p53, enhanced its transcriptional activity and induced apoptosis in a p53-dependent manner.

Discussion and conclusions: In the present study, we examined the interaction of NEDL1 with mutant SOD1s and characterized the function of NEDL1 in induction of apoptosis. NEDL1 is a quality control E3 ligase that recognizes mutant SOD1 to form a tight complex in motor neurons of FALS patients. Our findings suggest that NEDL1 may play a pivotal role in inducing neuronal cell death through functional interaction with mutant SOD1 and p53.

P171 HIGH INCIDENCE OF UBIQUITINATED INCLUSIONS IN AMYOTROPHIC LATERAL SCLEROSIS FROM AN AGED COHORT

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Background: Ubiquitinated neuronal intracytoplasmic inclusion (UI) is interpreted to be a hallmark of frontotemporal dementia with and without amyotrophic lateral sclerosis (ALS).

Objectives: We examined incidence of UI from an aged cohort and its pathological significance.

Materials and methods: We examined 1822 serial autopsy cases (average 80.7 years old, 974 men and 848 women) from a geriatric general hospital since 1995. Dentate gyrus was screened immunohistochemically with anti-ubiquitin antibody (polyclonal, Sigma-Aldrich, St. Louis, MO).

Results: UI was observed exclusively in nine (40.9% of total ALS, average age of 71.9 years, five men and four women) out of 22 ALS cases (average age of 74.3 years, eight men and 14 women) in the series. Seven out of the nine UI-positive (UI+) cases showed subicular degeneration and presented with clinical dementia rating (CDR) zero (two cases); 0.5 (one case); and equal to or above 1 (four cases), while two UI+ cases without subicular degeneration were categorized to CDR zero (one case) and CDR 1 (one case). In contrast, 13 UI-negative (UI−) cases were classified into CDR zero (11 cases) and CDR 0.5 (two cases). The total clinical course of UI+ cases was 29.2 months and showed no difference from 30.5 months of UI− cases.

Discussion and conclusion: Our study shows that UI is specifically and frequently present in ALS cases from an aged cohort and significantly contributes to their cognitive decline.

P172 NEURONAL INCLUSIONS IN SPORADIC MOTOR NEURON DISEASE ARE NEGATIVE FOR ALPHA-SYNUCLEIN

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Background: Little is known about the immunoreactivity of α-synuclein in the inclusions, such as Lewy body-like inclusions and Bunina bodies, observed in ALS/MND, and in particular, that in skein-like inclusions and basophilic inclusions is almost untouched.

Objective: To examine whether ALS/MND can be categorized as a α-synucleinopathy.

Methods: Using anti α-synuclein antibodies, we studied immunocytochemically those inclusions observed in anterior horn cells of ALS. The spinal cords of 28 autopsied sporadic ALS and one MND (with basophilic inclusions) patients, all of which were obtained within six h after death, were investigated (ages 47–83 years; average 65.0 years). Sixteen age-matched patients without any neurological disease served as controls (ages 35–81 years; average, 64.2 years). Lumbar spinal cords were immunostained with two antibodies to α-synuclein (a polyclonal antibody to amino acid residues 121–136: anti-human α-synuclein S122, IBL and a polyclonal antibody to amino acid residues 124–134 of human α-synuclein with phosphoserine 129: anti-PSer 129, a gift from Dr. T. Iwatsubo) and ubiquitin (polyclonal, rabbit, Dako). Immunoreaction was visualized by the streptavidin-biotin peroxidase complex method.

Results: Immunostaining with anti-PSer 129 did not produce background staining or anti-α-synuclein-immunoreactive spheroids, but intensely and specifically labeled Lewy bodies, Lewy neuritis and glial cytoplasmic inclusions. Immunostaining with anti-S122 produced background staining, but intensely labeled Lewy bodies, Lewy neuritis and glial cytoplasmic inclusions. Lewy body-like hyaline inclusions, skein-like inclusions, Bunina bodies, basophilic inclusions, or spheroids were not immunostained for α-synuclein (PSer 129 or S122), whereas Lewy body-like hyaline inclusions and skein-like inclusions were immunostained for ubiquitin. In the controls, these inclusions were not observed in the somata of the anterior horn cells or their neuronal processes.

Conclusion: Our findings do not support the hypothesis that ALS/MND could be classified as one of the diseases grouped as α-synucleinopathies.

P173 PROTEIN SYNTHESIZING SYSTEM IN THE MOTOR NEURONS IN THE SPINAL CORD IN AMYOTROPHIC LATERAL SCLEROSIS: PURSUING THE BEGINNING OF THE ALTERATIONS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive fatal disease involving the upper and lower motor neurons in human adults. Among the neuropathological findings, central chromatolysis (decrease of cytoplasmic RNA and rough endoplasmic reticulum (rER)), axonal accumulation of phosphorylated neurofilaments and fragmentation of the Golgi apparatus have been reported to be the early changes. However, inter-relationship or hierarchy of these findings and the other findings reported in the protein synthesizing system have not been elucidated.

Objectives: To pursue the beginning and to clarify the inter-relationship of the findings, the present authors examined: 1) the transcriptional activity of ribosomal (r)RNA gene, 2) amount of cytoplasmic RNA, 3) ultrastructural alteration of the rER, and 4) inter-relation of molecular chaperone (KDEL) and fragmentation of Golgi apparatus in the motor neurons in the spinal cord and brainstem.

Methods: 1. Patients with sporadic ALS, and experimentally, rats with the SOD1 gene mutation (H46R) and with facial nerve avulsion were used. 2. Transcription activity of the rRNA gene was measured in the motor neurons in the spinal cord in sporadic ALS, SOD1-transgenic (Tg) rats and in the motor neurons in the facial nerve avulsed rats by AgNORs (silver staining of nucleolar organizer region-associated proteins) using formalin-fixed paraffin-embedded sections. 3. Ultrastructural findings of the rER of the spinal motor neurons in sporadic ALS patients were examined. 4. Immunohistochemistry for KDEL and TGN 46 was performed.

Results: 1. Transcription activity of the rRNA gene decreased in the motor neurons in the spinal cord in sporadic ALS patients. 2. Transcription activity of the rRNA gene did not decrease in the motor neurons in the spinal cord in SOD1-Tg rats, although the rats showed marked depletion of the number of motor neurons. 3. Transcription activity of the rRNA gene decreased significantly in the motor neurons of the facial nucleus from 2 h after the avulsion, and the number of the neurons depleted from two weeks after the operation. 4. Ribosomal detachment on the rER was found in the spinal motor neurons in sporadic ALS patients. 5. Abnormal

localization of KDEL was found even in the spinal motor neurons showing normal-looking Golgi apparatus in sporadic ALS patients.

Discussion and conclusions: 1) Decrease of the transcription activity of rER in motor neurons is a sign of the very early stages of the degeneration of the motor neurons, and depletion for a certain period induces loss of the motor neurons. 2) Spinal motor neurons in the SOD1-Tg rats degenerate in a different way from the sporadic ALS patients. 3) Localization of molecular chaperon KDEL changes earlier than the fragmentation of the Golgi apparatus.

P174 GOLGI APPARATUS OF THE MOTOR NEURONS IN PATIENTS WITH ALS AND OTHER MOTOR NEURON DISORDERS

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Background: Several studies revealed that the Golgi apparatus (GA) of the anterior horn cells was fragmented in amyotrophic lateral sclerosis (ALS) patients and in asymptomatic transgenic mice expressing the G93A mutation of the gene encoding Cu/Zn SOD1. Therefore, GA is one of the early targets of the pathological processes initiating the neuronal degeneration in ALS.

Objectives: We investigated the GA of motor neurons in patients with sporadic ALS and other motor neuron disorders (MND) including familial ALS, juvenile ALS with basophilic inclusions (BIs) and X-linked spinal and bulbar muscular atrophy (SBMA). We also examined the relationship of any inclusions in MND and Golgi fragmentation.

Methods: Sections from ALS/MND including 16 cases of sporadic ALS, two cases of juvenile ALS with BIs, three cases of familial ALS with SOD1 mutations and five cases of SBMA were immunostained with anti-MG160, −SOD1 (Cu/Zn) and −1C2 antibodies.

Results: The GA in over 50% of the remaining anterior horn cells and in 13.2% of examined Betz cells was fragmented in the patients with sporadic ALS. In juvenile ALS with BIs, 60% of the preserved large motor neurons showed fragmentation of the GA. Ten of 14 preserved large motor neurons showed fragmentation and reduced numbers of GA. The GA of residual motor neurons in patients with SBMA was normal or reduced in size, and the profiles of small or atrophic GA were different from the fragmented GA. Double immunostaining using anti-MG160 and -1C2 antibodies showed that anterior horn cells bearing an intranuclear inclusion had a normal or atrophic GA. Fragmentation of the GA was not observed in neurons bearing an intranuclear inclusion. On the other hand, the GA of the majority of anterior horn cells containing Bunina bodies, BIs and SOD1-positive aggregates were fragmented or not strongly stained compared to normal GA.

Discussion and conclusions: We showed abnormal proteinaceous cytoplasmic aggregates may be related to the fragmentation of GA in patients with sporadic ALS, juvenile ALS and familial ALS; however, the fragmented GA was not found in patients with SBMA. The precise molecular mechanisms linking the aggregation of proteins and fragmentation of the GA are not known, and the identification of the molecular composition of the aggregated proteins present in patients with sporadic ALS with a variety of inclusions remains a major challenge.

P175 EVIDENCE FOR SYSTEMIC INFLAMMATION IN PATIENTS WITH ADVANCED AS COMPARED TO MILD AMYOTROPHIC LATERAL SCLEROSIS

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Background: Inflammatory processes have been described in the brain and spinal cord tissue in ALS. They may be protective to nervous tissues by inducing cell regeneration, but also injurious by causing tissue damage, thereby complicating the assessment of the significance of inflammation for the understanding of the disease, prognosis and treatment options.

Objectives: The aim of the present study was to determine blood levels of parameters of systemic inflammation in patients with amyotrophic lateral sclerosis (ALS) and to correlate findings to clinical variables.

Methods: We determined the intensity of inflammation in the serum of 50 patients with ALS (30 males) without any clinical systemic inflammatory diseases by measuring generally accepted parameters of inflammation (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen). Results were compared with the ALS functional rating scale (ALSFRS), which is a sensitive measure of disability.

Results: Patients were 60 ± 14 years old; their ALSFRS was 27 ± 9 (range 7–40). Mean ESR was 14.7 ± 10.7 (range 2–50), CRP was 2.9 ± 3.4 (range 0.04–16.6), fibrinogen was 323 ± 67 (range 184–470). ESR was abnormal in 13 patients; pathologic CRP was found in seven patients. Both ESR and CRP were correlated to ALSFRS ($p < 0.05$), with higher values in more disabled patients. Fibrinogen levels were normal in all patients and were not correlated to ALSFRS.

Conclusions: Although the measured parameters of inflammation were within accepted normal limits in most patients, there was a trend toward higher ESR and CRP in more disabled patients. It is not clear whether this finding is related to a basic inflammatory process in ALS or to disease-related systemic complications. We intend to

follow these patients in order to determine whether the relationship between clinical status and inflammatory measures is maintained over time and whether these simple routine tests might be of prognostic value.

P176 SERUM LEVELS OF SOLUBLE E-SELECTIN AND L-SELECTIN IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Immunological abnormalities have been implicated in amyotrophic lateral sclerosis (ALS) pathogenesis. Adhesion molecules are markers of activated endothelial cells up-regulated by cytokines.

Objectives: The aim of the present study was to investigate whether the vascular cells of ALS patients are activated.

Methods: We assayed repeatedly over a two-year period soluble E-selectin (sE-selectin) and soluble L-selectin (sL-selectin) in sera from 69 ALS patients (28 women and 41 men; mean age $\pm$SD: 62 ± 10 years). Disease severity was scored by means of the ALS Functional Rating Scale, and patients were subgrouped accordingly into three classes: I (scores between 40 and 31); II (scores from 30 to 11); III (between 10 and 0).

Blood samples were drawn in the morning, and serum was stored immediately at -20°C.

Adhesion molecules were measured by enzyme-linked immunosorbent assay (ELISA) (R&D systems).

Results: Presented selectin data relate to assays at time of diagnosis (T_0) and those at the time of the most recent clinical examination (T_n).

Mean sE-selectin levels were higher, but not significantly, in class I vs. class II vs. class III patients at T_o (45.4 ± 31.2, 38.3 ± 20.1, and 23.4 ± 3.1 ng/ml, respectively) and at T_n (34.2 ± 12.7, 30.1 ± 16.6, and 29.6 ± 15.9 ng/ml, respectively).

Mean sL-selectin levels were mostly in the normal range and did not differ significantly among patients' classes.

Discussion: Our data, although still preliminary, suggest a different role of sE-selectin and sL-selectin in ALS: the former might be down-regulated as disease progresses.

The precise meaning of these findings in terms of vascular cell activation is not clear. Mechanisms involved in such a modulation are currently largely unknown: further studies are much needed to confirm our data and shed light on related molecular events.

P177 THE ACTIVITY OF PROSTAGLANDIN E(2) IN CEREBROSPINAL FLUID FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by selective killing of upper and lower motor neurons. Neuroinflammation is thought to play a role in the pathogenesis of ALS, probably as an accompanying process, contributing to disease progression. Among several inflammatory mediators, prostaglandin E2 (PGE2) has been associated with ALS pathology. PGE2-induced injury, apart from its pro-inflammatory and pro-apoptotic mode of action, occurs through the calcium-dependent release of glutamate from astrocytes that leads to excitotoxic neuronal cell death. PGE2 has been found to be increased in cerebrospinal fluid (CSF) from ALS patients (1–3).

Objectives: To evaluate the possible involvement of PGE2 in ALS pathomechanism by measurement of the concentration of PGE2 in CSF of Polish ALS patients and comparison with non-ALS controls.

Methods: Twenty-one ALS patients (9 females, 11 males, aged 33–73 years, mean 55.19 ± 10.24 years) fulfilling WFN Criteria and 28 diseased, non-ALS controls (17 females, 11 males, aged 41.92 ± 17.39 years) were enrolled in the study. CSF was obtained from all patients using diagnostic lumbar puncture and stored at -70°C until assayed. The radioimmunoassay (RIA) method was used for PGE2 CSF analysis.

Results: We noticed lower PGE2 levels in CSF obtained from ALS patients than in controls, although the difference was not statistically significant (109.04 ± 132.24 pg/ml and 132.20 ± 114.68 pg/ml, respectively, $p > 0.05$). The mean ($\pm$SD) values of PGE2 level in CSF from limb onset and bulbar onset ALS patients were very similar: 107.86 ± 145.40 vs. 112.8 ± 90.16 pg/ml, respectively, $p > 0.05$. The mean ($\pm$SD) value of PGE2 level in CSF from ALS patients with predominantly lower motor neuron involvement was higher compared to patients with predominantly upper motor neuron involvement: 140.55 ± 143.33 pg/ml vs. 30.27 ± 44.71 pg/ml, and this difference was statistically significant ($p = 0.006$).

Discussion and conclusions: Our results do not confirm previous reports of increased levels of PGE2 in CSF from ALS patients. The reason for this might be a different method used for PGE2 measurement. We used radioimmunoassay, whereas former assessments were made by chemiluminescence immunoassay (1) or enzyme-linked immunoassay (2,3). The latter may give cross-reactions with particles other than PGE2 and therefore may be less

sensitive. Another reason for this discrepancy might be differences in duration of the disease between ALS groups studied: in our study average time of duration of the disease was 27.2 months, while in the previous studies it was 17 months (2) or 11.8 months (1). These results may suggest that PGE2 plays a major role in the earliest phase of ALS, which is in agreement with results of another study (3).

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P178 EVIDENCE FOR ABNORMAL MONOCYTE IMMUNOGLOBULIN RECEPTOR EXPRESSION IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS)

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Background: Immunoglobulins (IgG) from ALS patients have been shown to induce microglial activation and motor neuron degeneration in Balb/c mice (1,2) suggesting a pathogenic role for some form of IgG in ALS pathogenesis. In a recent study overall IgG levels were found to decrease with ALS disease progression; however, levels of CD16 (Fcγ receptor III) expression on activated blood monocyte/macrophages (MO) were found to be elevated (3). The systemic modulation of IgG levels and Fc receptors, critical for mediating phagocytosis in macrophages, suggests some critical interplay between macrophages and immune complexes in ALS; no systematic evaluation of Fc receptor expression in ALS has been performed and this was the subject of the current study.

Objectives: 1) To evaluate expression of Fc-gamma receptors, CD32 (Fcγ receptor II), CD64 (Fcγ receptor I), and CD89 (IgA receptor), on circulating MO in ALS patients and healthy controls. 2) To determine whether expression of immunoglobulin receptors correlated with clinical stage of disease or riluzole treatment in ALS.

Methods: Heparinized blood was obtained from 35 patients with SALS and 30 age-matched normal controls (N). Flow cytometry was performed to quantitate median fluorescence intensity (MFI) for expression of CD32, CD64, and CD89 on CD14+ MO. Results from immune studies were evaluated in relation to riluzole treatment and severity of neurological impairment as determined by the revised ALS Functional Rating Scale (ALSFRS-R).

Results: Patients with SALS had significantly elevated levels of CD64 (MFI CD14+CD64+: N, 88.1 ± 26.0; SALS, 121.8 ± 30.8, $p < 0.0001$) and CD89 (MFI CD14+CD89+: N, 113.7 ± 40.5; SALS, 148.4 ± 47.2, $p < 0.005$) expression on blood MO compared to controls. CD32 levels were similar between patients with SALS (MFI CD14+CD32+=21.1 ± 21.0) and control subjects (MFI CD14+CD32+=1 6.1 ± 16.8). Expression levels of CD64 and CD89 on MO were significantly elevated at all levels of SALS disease severity unrelated to stage of disease. Untreated SALS patients showed significantly higher CD89 MO expression (186.9 ± 44.0, $n=7$) compared to patients treated with riluzole (138.0 ± 43.4, $n=23$, $p < 0.05$) and normal controls (113.7 ± 40.5, $n=30$, $p < 0.001$). Untreated SALS patient MO had lower levels of CD32 than that of riluzole treated, but this difference did not reach statistical significance (untreated=9.3 ± 13.5, treated=24.0 ± 22.0, $p=0.107$). No difference between riluzole treated and untreated SALS patient MO expression of CD64 was observed, similar to previous findings of unchanged MO CD16 expression.

Conclusions: Patients with SALS showed persistent disease-associated increases in CD64 and CD89 expression on circulating MO in SALS, which confirms the earlier study of systemic immune alteration in ALS3. Riluzole use was associated with normalization of two MO immune activation parameters (CD89 and CD32), whereas three others (CD16, CD64 and HLA-DR) remained abnormal. Thus riluzole use, which extends the lifespan of treated patients, may function in part through modulating MO activation suggesting a potential role for MO targeted therapy in ALS.

P179 MONOCLONAL GAMMOPATHY IN PATIENTS WITH (RAPIDLY PROGRESSIVE) MOTOR NEURON DISEASE

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Background: Previous studies have reported higher frequencies of monoclonal gammopathy in patients with motor neuron disease, 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 immunoglobulin in serum.

Objective: To establish the frequency of monoclonal immunoglobulin in serum in rapidly progressive motor neuron disease (ALS and PSMA).

Methods: From 1 January 2001 to 1 July 2005, consecutive patients diagnosed with ALS ($n=274$) and PSMA ($n=5$) were screened for the presence of monoclonal immunoglobulin in serum by agarose electrophoresis and consequent immunofixation of suspected bands.

Results: Clinical characteristics of patients with ALS and PSMA were representative of those reported in previous population-based studies. Monoclonal immunoglobulin was present in sera of 5.5% of the ALS and 16.9% of the PSMA patients.

Conclusion: The frequency of monoclonal gammopathy in patients with PSMA is higher than in the general population. A recent population-based study showed that monoclonal gammopathy occurs in 3.2% of persons 50 years of age and older. After adjusting for age and sex, a positive association of monoclonal gammopathy with PSMA remains. (The highest age-adjusted rate (males age 80 and older) in the general population is 8.3%.) Moreover, monoclonal gammopathy occurs more frequently in PSMA than in ALS patients (16.9% vs. 5.5%).

P180 INVOLVEMENT OF TUMOUR NECROSIS FACTOR SYSTEM IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Many hypotheses have been formulated on the pathogenesis of amyotrophic lateral sclerosis (ALS): modifications of dismutase activity of SOD1 protein, aggregates of proteins causing proteasome impairment, excitotoxicity, and autoimmunity. The implication of the immune system in ALS is supported by data on activated T-cells in spinal cord and brain of sporadic (SALS) patients (1). Our previous findings demonstrated that SALS lymphocytes stimulated with a reactive oxygen species donor show traits similar to those of ALS motor neurons (2). Abnormal levels of interleukin-6 and tumour necrosis factor (TNFα) have been found in cerebrospinal fluid and sera from ALS patients (3). TNFα and its soluble receptor (sTNF-Rs) have been found at significantly higher levels in ALS patients' sera (4).

Objectives: The aim of our study was to assay TNFα system expression and describe its time course during disease progression.

Methods: Eighty-eight ALS patients diagnosed according to the El Escorial criteria were involved in this study. Blood samples from all patients were drawn every two months over six years: on 540 samples 533 TNFalpha, 501 sTNF-RI and 518 sTNF-RII assays were carried out by ELISA method using a commercial kit (Bender MedSystems, Vienna, Austria).

Results: TNFα, sTNF-RI and sTNF-RII levels were determined in each patient by calculating the mean values of all their assays over time. Such mean values were compared with the range of normal plasma values (TNFα: <14 pg/ml; sTNF-RI: 0.3–2.9 ng/ml; sTNF-RII: 1.9–8.5 ng/ml). TNFα levels were higher in 86.5% of SALS patients, whereas only 13.4% and 9.7% of SALS patients showed higher sTNF-RI and sTNF-RII levels, respectively. Interestingly, the time course of TNFα, sTNF-RI and sTNF-RII expression defined two patients' subgroups: the former had a well-defined typical time curve for both TNFα and its soluble receptors; the latter displayed a scattered value distribution over time. No correlation was found between the two groups and with any marker of the disease severity.

Discussion: Circulating levels of antigenic TNFα are significantly increased in plasma from ALS patients according to the published data (4). On the other hand, we did not find enhanced sTNF-Rs levels in our ALS population. A possible explanation could be that we considered a higher number of assays and included not only probable/definite ALS, but also possible ALS patients according to the El Escorial criteria. Our findings suggest that there might not be TNF system modulation in ALS and that increased amounts of TNFα might be a non-specific inflammatory response.

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P181 CHANGES IN GRANULOCYTE COLONY STIMULATING FACTOR AND ITS RECEPTOR IN THE CNS OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Neuro-glial inflammation is being paid keen interest in the study of ALS pathogenesis. In particular, cytokines/chemokines may play an important role in motor neuron cell death through immune-mediated neuro-glial inflammation.

Objectives: The aim of this study was to characterize multiple cytokine profiles in ALS cerebrospinal fluid (CSF) using multiplexed fluorescent bead-based

immunoassay and to clarify the biological significance of relevant cytokines through immunohistochemical analyses of autopsied ALS spinal cords and cell culture assays.

Methods: We simultaneously measured 16 cytokines/chemokines (interleukin (IL)–1β, IL–2, IL–4, IL–5, IL–6, IL–7, IL–8, IL–10, IL–12 (p70), IL–13, IL–17, interferon–γ, tumor necrosis factor-α, granulocyte colony stimulating factor (G-CSF), macrophage chemoattractant protein-1 (MCP–1) and macrophage inflammatory protein-1β) in CSF and sera from 37 sporadic ALS patients and 33 controls using a multiplexed fluorescent bead-based immunoassay, a recently developed powerful technology. We also conducted immunohistochemical analyses of the spinal cords from eight autopsied ALS cases and six non-neurological disease controls as well as cell culture analyses of relevant cytokines and their receptors.

Results: We found that concentrations of G-CSF and MCP-1 were significantly increased in ALS CSF compared with controls, but not sera, compared with controls. In the autopsied spinal cords, G-CSF was expressed in reactive astrocytes in ALS cases but not controls, whereas G-CSF receptor expression was significantly decreased in large motor neurons of spinal cords from ALS cases as assessed by densitometry. Biologically, G-CSF had a protective effect on the motor neuron cell line NSC34 under conditions of both oxidative and nutritional stress.

Conclusion: We consider that G-CSF has a potentially neuroprotective effect on motor neurons in ALS and that down-regulation of its receptor might contribute to ALS pathogenesis.

P182 INCREASED IL-13 BUT NOT IL-4 PRODUCTION BY CD4-POSITIVE T-CELLS AND CD8-POSITIVE T-CELLS IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that selectively destroys both upper and lower motor neurons. Although the etiology of the disease is still not clearly understood, recent reports suggest that neuroinflammation may be involved in motor neuron degeneration.

Objective: The aim of this study was to investigate the involvement of immune mechanisms in the pathogenesis of ALS by measuring the intracellular cytokine production profile of peripheral blood CD4+ and CD8+ T-cells.

Methods: Twenty-one ALS patients and 16 healthy controls were enrolled in this study. Peripheral blood mononuclear cells were treated for 4 h with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin A. After staining the surface antigens, cells were permeabilized and stained for the intracellular cytokines. Finally, the cells were analysed by three-color flow cytometry using Epics XL System II. In this study, intracellular production of Th1 cytokines (IFN-γ, TNF-α and IL-2), and Th2 cytokines (IL-4, and IL-13) in peripheral blood CD4+ and CD8+ T-cells was measured. We also evaluated the severity of the disease using the ALS Functioning Rating Scale (ALS FRS).

Results: The ALS patients showed significantly higher CD4+IL-13+ and CD8+IL-13+-cell percentages than controls ($p < 0.05$). However, the same group showed no significant change in levels of either Th1 cytokines, or the number of IL-4-producing cells. In addition, there was a negative correlation between CD4+IL-13+ cell percentages and ALS FRS.

Discussion and conclusion: We found here a selective up-regulation of IL-13 producing cells in ALS patients. As IL-13 upregulates MCP-1 expression in monocytes and microglias, increased IL-13 producing cells may contribute to the disease process of ALS, as up-regulation of MCP-1 has been reported in CSF.