Abstracts from Theme 9
Scientific and Clinical Work in Progress & Care Practice

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Contents Page

SCIENTIFIC WORK IN PROGRESS.................................................. 4

P171 GLUR2 AMPA RECEPTOR SUBUNIT EDITING IN SPINAL MOTOR NEURONS 4
   Figlewicz DA, Mobley B, Hong Y ................................................................. 4
P172 A NOVEL TOOL FOR IDENTIFYING PROTEINS AND COMPOUNDS
   THAT INHIBIT OR ACCENTUATE THE PATHOGENIC PROCESS IN
   A MODEL OF ALS ................................................................. 5
   Wright PD¹, Tuite MF², Shaw CE¹ ............................................................ 5
P173 DECREASED GLUR2 EXPRESSION IN AGGREGATE-BEARING MOTOR NEURON
   CULTURES DOES NOT LEAD TO INCREASED SUSCEPTIBILITY TO CELL DEATH 6
   Sanelli T¹,², Ge W², Strong M¹,² ................................................................. 6
P174 TEMPORAL PROFILES OF NEURONAL DEGENERATION, GLIAL PROLIFERATION,
   AND CELL DEATH IN HNFL+/+ AND NFL/-/ MICE............................... 7
   McLean JR¹,², Sanelli T¹,², Yang W², Leystra-Lantz C², Robertson J³, Strong MJ¹,²* 7
P175 ISOLATION AND CHARACTERISATION OF INSOLUBLE HSOD1 PROTEIN SPECIES
   IN THE G93A SOD1 MOUSE. .............................................................. 9
   Banner SJ¹, Dunn MJ³, Miller CJ³, Shaw CE¹,² ....................................... 9
P176 EVOLUTION OF CELLULAR AND MOLECULAR PATHOLOGY IN A HUMAN
   MUTANT SOD 1 TRANSGENIC MOUSE MODEL OF MND..................... 10
   Moisse K¹,², Maekawa S¹,², Al-Sarraj S¹, Banner S², Shaw C², Leigh PN² 10
P177 QUANTIFICATION OF GENE EXPRESSION IN THE PRECENTRAL AND ANTERIOR
   FRONTAL REGIONS IN MOTOR NEURONE DISEASE ......................... 11
   Maekawa S, Al-Sarraj S, Leigh PN ...................................................... 11
P178 EPIGENETIC REGULATION OF HUMAN NEURAL STEM CELL DIFFERENTIATION 12
   Miller TM, Flax JD, Futscher B, Watts G, Imrie D ................................ 12
P179 FINDING NEW GENES CAUSING AMYOTROPHIC LATERAL SCLEROSIS 13
   Gopinath S, Kennerson M, Nicholson GA ......................................... 13
P180 AN AUTOPSY STUDY ON THREE PATIENTS CARRYING THE D90A SOD1
   MUTATION ......................................................................................... 14
   Graffmo K¹, Jonsson A¹, Marklund SM¹, Andersen PM², Brannstrom T¹ 14
P181 SMN IN THE LIFE AND DEATH OF MOTOR NEURONS .............. 15
   Leach K, Kerr D, Yin D, Hardwick JM .................................................. 15
P182 ADULT MOTONEURONE RESCUE WITH AN IGF-1 SPLICE VARIANT (MGF)
   DERIVED FROM MUSCLE .............................................................. 16
   Johnson IP¹ Aperghis MA² Goldspink G² ............................................. 16
P183 FROM ORIGINAL CELL-BASED ASSAYS TO NOVEL THERAPEUTICS FOR MOTOR
   NEURON DISEASES ..................................................................... 17
   Abitbol J-L ...................................................................................... 17

CLINICAL PRACTICE AND WORK IN PROGRESS ............... 18
P184 A CASE OF FAMILIAL ALS WITH MARKED ASYMMETRY........18
   Probst A¹, Andersen P², Weber M³...............................................................18

P185 AUTONOMIC DYSFUNCTION OF THE GASTROINTESTINAL SYSTEM IN
AMYOTROPHIC LATERAL SCLEROSIS: A CASE STUDY..................19
   Choudry R, Croul S, Deboo A, Heiman-Patterson TD..........................19

P186 THE NATIONAL REGISTRY OF US VETERANS WITH ALS........20
   Kasarskis EJ, Dominick K, Oddone EZ..................................................20

P187 BEHAVIOURAL ASSESSMENT OF THE DYSEXECUTIVE SYNDROME:
NEUROPSYCHOLOGICAL STUDY IN PATIENTS WITH AMYOTROPHIC LATERAL
SCLEROSIS. ........................................................................................................21
   Piquard A, Salachas F, Derouesné D, Lacomblez L, Meininger V...........21

P188 PREFERENCES OF PATIENTS WITH ALS FOR ACCURATE PROGNOSTIC
INFORMATION ....................................................................................................22
   Armon C¹,², Schultz JD ..............................................................................22

P189 RESPIRATORY MANAGEMENT OF PATIENTS WITH MND / ALS, AN MND CARE
CENTRE EXPERIENCE ..............................................................................23
   Clarke J, Orrell RW, Howard RS ...............................................................23

P190 “HOW TO COMMUNICATE IF ONE OF THE PARTNERS CAN NOT SPEAK” 24
   Lunde FL, Kulo LS, Pfaff K.........................................................................24

P191 “HOW TO EXPRESS YOURSELF WHEN SPEECH IS LOST”.......24
   Lunde F, Kulo LS, Pfaff K.........................................................................24

P192 SOCIAL LAW PROVIDES HOME MECHANICAL VENTILATION ..26
   Rasmussen Barrutia BRB, Lorentzen CK, Trojel DT..............................26

P193 AMYOTROPHIC LATERAL SCLEROSIS BRAZILIAN ASSOCIATION (ABRELA):
SOCIAL WORK REPORT. ..............................................................................27
   Fernandes E, Macario MF, Quadros AAJ, Silva HCA, Oliveira ASB......27

P194 BREAST CANCER IN WOMEN WITH MOTOR NEURON DISEASE28
   Viader F¹, Corcia P², Carluer L¹, Sadot E¹, Levy C³, Delozier Y³ ..........28
Background
Glutamate-receptor mediated neuronal death is believed to result from influx of Ca\(^{2+}\) following receptor activation, and our studies have shown that either blockade of Ca\(^{2+}\) permeable AMPA receptors or induced expression of the cytoplasmic Ca\(^{2+}\) binding protein Calbindin-D28K can rescue motor neurons from several categories of lethal stress. Ca\(^{2+}\)-influx through the AMPA receptor is blocked when at least one of the four possible subunits of the receptor is GluR2, whose mRNA is edited at a specific basepair. As a result of RNA editing, an arginine residue replaces the coded glutamine residue ("Q/R editing") to cause this blockade. Under control conditions, this editing event modifies >99% GluR2 mRNA. We hypothesize that stress of motor neurons leads to a decrease in GluR2 subunit mRNA editing and, hence, to increased synthesis of the Ca\(^{2+}\)-permeable GluR2 AMPA receptor subunits.

Objectives
We are carrying out quantitative analysis of the expression level of the four major AMPA receptor subunits – GluR1, R2, R3, and R4— as well as the expression level of edited vs. unedited GluR2; the expression level of the editing enzyme adenosine deaminase (ADAR2); and the activity of ADAR2, in spinal motor neurons.

Methods
In order to be certain that only motor neurons are being studied we will employ single cell laser-capture followed by quantitative RT-PCR, subunit or isoform specific PCR and restriction digest, electrophoresis and imaging to establish the pattern(s) of synthesis of AMPA receptor subunits in motor neurons. Motor neurons laser-captured from the spinal cord of the mutant SOD (G93A)-overexpressing transgenic mice will be examined at various disease stages (presymptomatic vs. early disease vs. endstage).

Results
Work in progress.

Discussion and conclusions
Most studies to date have employed methodologies which have limited the usefulness of the data generated. For example, studies using human postmortem tissue to study expression of the GluR subunits are limited by RNA degradation during the postmortem delay in tissue isolation, and also by the fact that what is being examined is end-stage disease and cannot shed much light on the
disease process. Animal studies using freshly isolated spinal cord are limited by the fact that many spinal cell types are being studied whereas motor neurons comprise only a fraction of the total material. Immunocytochemical studies of AMPA receptor subunits in spinal cord can provide data which are motor neuron specific, but these studies at the level of the protein cannot determine whether or not the GluR2 subunit has been edited. Our aim has been to design the studies of GluR subunit expression using methodologies which circumvent these limitations.
P173 DECREASED GLUR2 EXPRESSION IN AGGREGATE-BEARING MOTOR NEURON CULTURES DOES NOT LEAD TO INCREASED SUSCEPTIBILITY TO CELL DEATH

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**Background**
We have previously shown that the co-localization of nNOS with neurofilamentous (NF) aggregates \textit{in vitro} in primary motor neurons cultured from \textit{hNFL +/+} transgenic mice is associated with the deregulation of calcium entry via the NMDA receptor. This calcium deregulation also resulted in increased apoptosis. AMPA receptors have also been implicated as players in the excitotoxicity theory of ALS, with the GluR2 subunit having a key role, as its absence confers calcium permeability to the AMPA receptor.

**Objectives**
The purpose of the present studies was to examine the role, if any, of the AMPA receptor in mediating calcium influx, which could lead to apoptosis, in our cell model. The two main objectives are: 1. To examine the expression of calcium-permeable AMPA receptors in \textit{hNFL+/-} versus C57 Bl/6 (control) motor neurons and 2. To determine the functional implications of calcium-permeable AMPA receptor expression in our cell culture model.
Methods
Day 14 post-plating (D14) motor neurons were fixed and stained for GluR1 (1:200; polyclonal rabbit, Chemicon) and GluR2 (1:200, monoclonal mouse, Chemicon), using fluorescent secondary antibodies (1:1000, goat anti-rabbit AF 546, and 1:250, goat anti-mouse AF 488, Molecular Probes) and confocal imaging. For Real Time (RT) PCR, total RNA was collected from D14 cultures, reverse transcribed and mRNA was amplified using probes for murine CHAT, GluR1, GluR2 and GAPDH. Live cell calcium imaging was performed using both Oregon-Green Bapta-AM and Fura-2-AM (Molecular Probes) calcium dyes. For apoptotic studies, D14 neurons were treated with increasing concentrations of glutamate (0-250μM) with or without glutamate receptor antagonists (10 μM CNQX or MK-801) for 1 hour in exposure buffer, which was subsequently replaced by serum-free media for 12 hours prior to cell fixation and staining for active caspase-3 (1:500, polyclonal rabbit, BD-Pharmingen) or ANNEXIN V.

Results
1. GluR2 was decreased at both the mRNA and protein level in hNFL+/+ cultures versus control.
2. There was no appreciable calcium influx via the AMPA receptor, nor any differences in calcium influx between hNFL+/+ and control cultures. 3. hNFL+/+ neurons were susceptible to glutamate-induced apoptosis at lower glutamate concentrations versus control. 4. The addition of AMPA receptor antagonist, CNQX, did not rescue hNFL+/+ cultures.

Conclusions
Although hNFL+/+ cultures express calcium-permeable receptors, they are not responsible for calcium deregulation in our culture system, nor do they play a major role in apoptosis. This does not rule out the possibility of an NMDA/AMPA receptor interaction, having a role in excitotoxicity in this aggregate-bearing cell model.

P174 TEMPORAL PROFILES OF NEURONAL DEGENERATION, GLIAL PROLIFERATION, AND CELL DEATH IN HNFL+/+ AND NFL-/− MICE.

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Background
Several animal models have been developed in an attempt to explain the mechanisms that mediate motorneuron degeneration in ALS. Two such models are found in mice overexpressing or lacking the neurofilament light subunit (NF-L). While most studies involving these two transgenic lines have focused on early axonal morphology, the developmental characterization of nonneuronal cell populations and neurodegenerative elements remains unknown.
Objective
To characterize temporal profiles of neuronal degeneration, glial proliferation, and cell death in hNFL+/+ and NFL-/- mice from 1 to 18 months.

Methods
Paraffin-embedded sections of C57bl/6, hNFL(+/+), and NFL(-/-) mice were examined at intervals of 1, 2, 4, 6, 9, 12, 15, and 18 months. Immunostaining was performed for neurofilament (polyclonal anti-phosphorylated NFH; 1:1000; NFH200, Sigma), microglia (polyclonal anti-IBA-1; 1:500; Wako Chemicals), astrocytes (monoclonal anti-GFAP; 1:1000; Pharmingen), activated caspase-3 (polyclonal cleaved caspase-3; 1:200; Cell Signaling), and HSP-70 (monoclonal anti-HSP-70; 1:100; Santa Cruz). Lumbar anterior horn cells were imaged by confocal or light microscopy and analyzed using Graphpad Software ANOVA with Tukey’s post hoc.

Results
No neurofilamentous (NF) aggregates were found in C57bl/6. NF aggregates were maximal in NFL(-/-) by 4 months and by 2 months in hNFL(+/+), both declining in numbers thereafter. By 18 months, remaining aggregates were localized to large motorneurons. There was a marked increase in the number of activated microglia from 4 to 12 months in both hNFL(+/+), and NFL(-/-) (p<0.01). By 18 months, both transgenic mice showed identical microglial staining with no evidence of activation. In contrast the astrocytic response was first evident by 9 months, maximal by 12 months (p<0.001), and persisted thereafter in NFL(-/-). A concomitant change in astrocytic structure accompanied these observations, characterized by an increase in the number and size of astrocytic processes. No significant differences were observed between hNFL(+/+) and C57bl/6. Apoptotic motorneurons were found in NFL(-/-) by 12 months (p<0.05) and persisted to 18 months, whereas hNFL(+/+), were first observed at 15 months (p<0.001). Caspase-3 staining was selective to large motorneurons in NFL(-/-) and to smaller motorneurons in hNFL(+/+). Increased HSP-70 immunoreactivity was observed in motorneurons from 1-4 months (p<0.01) and in glial cells at 12 to 18 months in NFL(-/-).

Conclusion
This study demonstrates unique time-specific patterns of neuronal degeneration, microglial, and astrocytic activation in two different transgenic models of neurofilamentous degeneration, only one of which demonstrates increased HSP-70 expression (NFL-/-). These studies have relevance to understanding the neuropathology of ALS.
P175 ISOLATION AND CHARACTERISATION OF INSOLUBLE HSOD1 PROTEIN SPECIES IN THE G93A SOD1 MOUSE.

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Motor neuron disease (MND) is an age-dependent neurodegenerative disorder that causes motor neuron degeneration, paralysis and death. Mutations in Cu, Zn superoxide dismutase (SOD1) are one cause for the familial form of this disease and mice expressing mutant SOD1 also develop paralysis. The mechanism whereby mutant SOD1 induces motor neuron degeneration is not understood, although it is generally accepted that mutant SOD1 acts via a toxic gain of function rather than a loss of dismutase properties.

Two hypotheses have been advanced to explain SOD1 mediated neuronal degeneration: aggregation and/or catalysis. Our laboratory has interests in both areas; however, here we focus more upon the aggregation potential of mtSOD1 molecules in relationship to disease.

Using the Gurney mouse model of MND (SOD1-G93A mutation) we have begun to investigate the proteomic profiles of various components of the mouse CNS at particular time points throughout the disease course. Electrophoretic investigations of mouse tissues confirm the presence of aggregating / insoluble SOD1 species within symptomatic animals. These SOD1 positive aggregating protein species are more prominent in spinal cord than within brain tissues and further analysis of the insoluble component of these tissues using 2D-gel electrophoresis has revealed the existence of numerous protein species besides SOD1. We hypothesise that SOD1 immunopositive aggregates are not composed solely of SOD1 molecules but are associated strongly with other proteins.

We are currently identifying and characterising the novel and differentially regulated proteins that are found within the insoluble fractions of the symptomatic G93A mouse CNS. It is hoped that the once identified this data may enable us to understand more fully the interactions that mtSOD1 has in vivo that ultimately result in degeneration.
P176 EVOLUTION OF CELLULAR AND MOLECULAR PATHOLOGY IN A HUMAN MUTANT SOD 1 TRANSGENIC MOUSE MODEL OF MND

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Background
Motor neuron disease (MND, amyotrophic lateral sclerosis) is a progressive neurodegenerative disease characterized by the selective vulnerability of upper and lower motor neurons. Mutations in the copper/zinc dismutase gene SOD1 are found in 20% of familial MND cases. Transgenic mice expressing human SOD1 with a point mutation at codon 93 causing a substitution from glycine to alanine (G93A) develop a motor neuron disease phenotype, while transgenic mice expressing wild-type human SOD1 do not.

Objectives
Several pathological features are observed in post-mortem tissue from human patients with MND resulting from SOD1 mutations. This study investigated whether such features are observed in susceptible neuronal populations in tissue from human mutant SOD1 transgenic mice and not in that from human wild-type SOD1 transgenic mice.

Methods
Frozen tissue was examined using luxol-fast blue Nissl and immunostaining for ubiquitin, astrocytes (GFAP), microglia (F4-80), a phosphorylated tau epitope (CP 13), and phosphorylated neurofilaments (SMI 31). This study further investigated the timing of these pathological changes in relation to the development of clinical disease symptoms through analysis of tissue at a presymptomatic time point (6 weeks), an early symptomatic time point (10 weeks) and a disease end stage time point (16-17 weeks).

Results
Perikaryal neurofilament phosphorylation and microglial activation were the earliest pathological changes, observed presymptomatically (P < 0.05) in MND cases compared with controls. These features became more widespread and intense throughout the disease course (P < 0.025). Other changes including neuronal loss, reactive astrogliosis, and perikaryal tau phosphorylation were not significant until disease end stage (P < 0.01), but exhibited an increasing trend throughout the disease course (P < 0.05). The presence of ubiquitinated inclusions was late event in the disease course, appearing in end stage disease tissue of 16-17 weeks (P < 0.01). Four areas considered susceptible to MND degeneration were investigated in this study: layer V of the motor cortex; the hippocampus; the facial motor nucleus of the brainstem; and the ventral horn of the spinal cord. Although pathological changes were observed in all these areas, they generally appeared earlier and were more pronounced in the lower motor neurons of the brainstem and particularly the spinal cord.
Conclusions
These results indicate that the human mutant SOD1 transgenic mouse does represent a valid model of MND both behaviourally and pathologically. A novel observation is that lower motor neurons in end stage MND cases were immunoreactive for CP 13, an antibody to tau phosphorylated at serine202. The observation of pathology before symptom onset in this model has therapeutic implications, suggesting the possibility of effectiveness of interventions targeting early, presymptomatic abnormalities.

P177 QUANTIFICATION OF GENE EXPRESSION IN THE PRECENTRAL AND ANTERIOR FRONTAL REGIONS IN MOTOR NEURONE DISEASE

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Background
Motor neurone disease (MND) brains are characterized by selective neuronal degeneration. Studies using antibodies against calcium binding proteins (calbindin-D28K,CB; parvalbumin, PV; calretinin, CR) to examine different populations of GABAergic interneurones and SMI-32 to examine glutamatergic neurones have shown that densities of calbindin-D28K-immunoreactive (-IR) and SMI-32-IR neurones in the primary motor, prefrontal, and anterior cingulate cortex in MND subjects are significantly decreased compared to controls. Reductions in the densities of PV-IR and CR-IR neurones in MND subject were not significant. The present study used quantitative RT-PCR to determine whether gene expression was altered in GABAergic interneurones from MND brains.

Methods
mRNA expression of CB, PV, CR, glutamic acid decarboxylase (GAD) 65 and 67 in the severely affected precentral gyrus (PCG) and the less severely affected extra-motor brain area, anterior frontal gyrus (AFG) were studied in brains from MND patients and control subjects using quantitative RT-PCR assay (TaqMan).

Results
Expression of CB mRNA was decreased (p = 0.04) and that of PV mRNA increased (p < 0.001) in the PCG of MND brains compared to control. There were no differences in the expression of CR, GAD65, GAD67, and GAD67 mRNAs.

Conclusions
mRNA and protein levels of CB are decreased in MND brains to controls. The cause of increased PV mRNA expression in MND brains is not clear but may be a compensatory mechanism.

Background
Epigenetic regulation is the stable activation/repression of gene expression with DNA methylation a critical mediator, occurring on cytosine bases in CpG dinucleotides. CpG motifs cluster at high density in "islands" that lie in the 5' region (encompassing the promoter) of ~60% of all genes. A large body of evidence indicates that methylation of CpG Islands leads to stable transcriptional repression of associated genes. Two mechanisms for this repression include: (1) a methyl-CpG steric inhibition of transcriptional activator binding; and (2) methyl-CpGs recruitment of methyl-CpG binding proteins (MBPs), which in turn recruit mediators of chromosome condensation and transcriptional repression.

Objective
To determine the methylation status of promoters in key neuronal and glial lineage-determining genes in neural stem cells (NSCs) and their differentiated progeny. We hypothesize that NSCs capacity for multi-potentiality and lineage commitment is mediated in part by epigenetic mechanisms. Using microarray technology, we quantitatively measure epigenetic state by determining the DNA methylation status of the promoters in genes known to establish glial vs. neuronal fate, as well as some downstream targets of these genes. In order to assess how these epigenetic changes effect gene expression, we compare the epigenetic state of these genes with their transcriptional level using Affymetrix microarrays.

Methods
Undifferentiated human NSCs derived from cortical regions of 16-20 month old fetuses (Cambrex Bioproducts) are expanded in vitro and harvested at time 0 (undifferentiated NSCs) and after 2 and 7 days of differentiation (early and intermediate time points of lineage commitment). Methylation status will also be assessed in mature neurons and astrocytes. In order to ensure statistical significance, three repetitions per group are performed for methylation and confirmatory gene expression analysis. RNA and DNA are simultaneously isolated from cells using a Qiagen RNA/DNA isolation kit. RNA expression is assessed by the Affymetrix Human Genome U1333 Plus 2.0 array and analyzed by NetAffx software. Methylation analysis utilizes linkers added to restriction enzyme digested genomic DNA. Fragments are separated into two pools; the first untreated and the second cleaved using the DNA methylation dependent (but sequence independent) McrBC restriction enzyme. Using linker-specific primers both pools are amplified and labeled via PCR, and used to probe a microarray that is spotted with a library of ~17,000 human CpG islands. Only unmethylated CpG islands give a signal on the array. These results are used to establish a quantitative estimate of methylation state at each CpG island, constituting a global methylation data set. This data set allows the examination of signal transduction pathways.
and their transcriptional targets, which regulate; proliferation and self-renewal, regional patterning, and glial-neuronal fate choice. Confirmation of CpG methylation results will be performed on 10 target genes by bisulfite sequencing with real time PCR to verify Affymetrix mRNA results.

Results
Experimental data will be reported.

P179 FINDING NEW GENES CAUSING AMYOTROPHIC LATERAL SCLEROSIS

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Background
The only definite causes of amyotrophic lateral sclerosis (ALS) are genetic. The genes causing familial ALS are associated with varying disease penetrance, hence less penetrant gene mutations may not be recognised as causing familial disease. Combined effects of genes causing ALS may be involved in sporadic ALS cases.

Objectives
1. To find new genes that singly or in combination cause familial ALS using linkage analysis.
2. To examine ALS families (SOD1 negative), for linkage studies in order to map additional motor neurone disease causing genes and loci.
3. To store and bank DNA samples (and transformed lymphocyte cell lines) from families, for future research.

Methods
We have been recruiting families for genetic studies for the past 10 years. In our ALS database, we have 166 families, of which 77 are known to be SOD1 negative families and 16 are SOD1 positive families.
Our SOD1 negative families are being expanded, as shown.

<table>
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</table>

All our families with ALS (who lack the SOD1 mutation) show autosomal dominant inheritance, and are being tested for autosomal dominant ALS loci by linkage and/or haplotype analysis. For
affected and deceased individuals whose DNA samples are unavailable, we have reconstructed the haplotypes with spouse’s and children’s DNA.

Results
Our largest familial SOD1 negative ALS family with 4 affected DNA samples has a linkage score (Zmax) 1.4 on simulation analyses. We have been able to exclude all four loci for autosomal dominant ALS in this family. Exclusion data will be shown.

Discussion
We have been able to exclude 4 ALS (autosomal dominant) loci in our largest family, confirming the genetic heterogeneity seen in ALS families. The variation of penetrance in ALS families poses the question of sporadic ALS being an autosomal recessive condition with low penetrance. Finding new genes in causing familial ALS can help to look for genes in causation of sporadic ALS. Most SOD1 negative ALS families are small with a low disease penetrance, making linkage studies difficult to perform. International collaborative efforts will help track the origin of small families and greatly facilitate their study.

P180 AN AUTOPSY STUDY ON THREE PATIENTS CARRYING THE D90A SOD1 MUTATION

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Background
Three patients carrying the D90A mutation in the SOD1 gene where identified from blood samples analysed at the ALS clinic at Umeå University Hospital. All patients had a slowly progressing motor neuron disorder characteristic of the D90A patients earlier described (Andersen et al). One of the patients having end stage disease died of a respiratory infection while another also died in respiratory tract infection even though being earlier in the disease process.

Objectives
The aim of the present investigation was to characterize the histopathological changes in the CNS accompanying the development of the disease.

Methods
Tissue from central and peripheral nervous system were obtained at autopsy with the prior consent of patient and relatives. Frozen tissue were subjected to analysis of content and activity of the SOD isoenzymes and formalin fixed paraffin embedded tissue were subjected to extensive histopathological and immunohistochemical studies.
Results
In all three cases small somatic superoxide dismutase 1 containing inclusions could be found. Ongoing studies are directed at localising these inclusions.

Discussion and conclusion
The study shows that the D90A mutation results in the accumulation SOD1 positive inclusions in both motor neurons of the spinal cord.

P181 SMN IN THE LIFE AND DEATH OF MOTOR NEURONS

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Alterations in the survival of motor neurons (SMN) gene that result in decreased production of full-length SMN protein cause spinal muscular atrophy (SMA). SMA is characterized by progressive degeneration of alpha motor neurons in the spinal cord, leading to muscle weakness and atrophy. Severe forms of SMA lead to early childhood death. SMN pre-mRNA can be alternatively spliced to yield mRNAs containing all coding exons or mRNAs lacking exons 7 or 5, which results in production of a truncated protein. Our laboratory has shown that full-length SMN proteins are capable of protecting differentiated neurons from apoptotic stimuli, whereas SMN proteins lacking sequences derived from exon 7 (SMND7) have lost protective activity and also increase neuronal death. Both the protective and pro-apoptotic activities of SMN variants were shown to be specific to differentiated neurons, as they were not observed in non-differentiated cells. These findings, therefore, indicated novel neuron specific functions for SMN.

In the present study, transfection of rat primary cortical neurons is used to demonstrate that SMND7 results in neuronal cell death. The reduction in neuronal viability observed upon expression of SMND7 occurs even in the absence of an additional apoptotic stimulus. Preliminary evidence also indicates that some common apoptosis related proteins are involved in this process. Further studies are underway, and are designed to further define the biological process through which SMND7 causes neuronal cell death.
P182 ADULT MOTONEURONE RESCUE WITH AN IGF-1 SPLICe VARIANT (MGF) DERIVED FROM MUSCLE

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Objectives
We have found that skeletal muscle expresses two main splice variants of Insulin-Like Growth Factor-1 (IGF-1). One of these (IGF-1Ea), is the same as the systemic or liver-type IGF-1 that is commonly used in experimental studies of neuroprotection. The other splice variant, mechano growth factor (MGF), is expressed by muscle in response to mechanical signals. The presence of a second isoform derived from the peripheral targets of motoneurones suggests MGF may be more suited to mediating local motoneurone-target interactions than a systemic isoform of IGF-1 that is derived mainly from liver. Here we compare the ability of liver-type IGF-1 and MGF to rescue mature motoneurones from death.

Methods
Facial motoneurone death was induced by nerve avulsion in 6 month-old rats (n=6 per group). 7 days prior to avulsion the snout was injected with i) plasmid cDNA containing functional copies of the IGF splice variants or ii) liver-type IGF-1 or MGF c-terminal peptide. In another group, gel-foam soaked in MGF c-terminal peptide was applied to the stylomastoid foramen immediately after avulsion. Motoneurones were quantified stereologically 1-3 months later.

Results
Avulsion resulted in 75-82% loss of motoneurones by 1 month. This loss was reduced at 1 month by prior injection of muscle with cDNA for liver-type IGF-1 (53%) or MGF (21%). This protection was largely lost by 3 month. Prior injection of muscle with liver-type IGF-1 or MGF c-terminal peptide did not reduce motoneurone loss at 1 month. However, application of gel foam soaked in MGF c-terminal peptide to the stylomastoid foramen at the time of injury reduced motoneurone loss to 50% at 1 month.

Conclusions
The level of neuroprotection provided by MGF cDNA is twice that of liver-type IGF-1. Our results indicate that presence of MGF at the time of avulsion is necessary, and that continued exposure prior to injury enhances MGF neuroprotection. Interestingly, the ability to up regulate MGF by exercise in humans is lost in old age. MGF may have a different mechanism of action to liver-type IGF-1 as the altered amino acid sequence of MGF indicates it has no glycosylation sites, while liver-type IGF-1 is glycosylated, and antibody block of the IGF-1 receptor does not block the myotrophic and neuroprotective effects of MGF. Use of MGF in neurological disease has been patented by UCL.
TROPHOS is a drug discovery and development biotech focused on identifying therapies for neurodegenerative disorders. Despite the identification of specific genes and proteins that are invariably associated with neurodegenerative disorders, there are currently no validated molecular targets and no effective therapies to slow or cure these devastating diseases. TROPHOS has developed a phenotypic screening approach that attempts to mimic the clinical situation. We have developed a number of cell based assays using primary motoneurons to identify drug candidates for ALS and spinal muscular atrophies (SMA). We consider primary neurons as a cellular test tube filled with the diverse products of 30,000 genes expressed under the conditions that are as close to the physiological environment as possible. Because this "black box" approach presents a formidable challenge to chemists who need structure activity relationships to optimize drug candidates, most drug discovery is based on identified and validated molecular targets. However selecting molecular targets one-by-one and validating them by trial and error is time consuming and offers no guarantee of success, TROPHOS' screening strategy focuses on the desired endpoint - neuronal survival - is a "fast track" to the identification of neuroprotective compounds that are stable, non-toxic and cross the cell membrane, if necessary, to get to their target (or targets) to keep neurons alive.

TROPHOS has successfully applied this approach to screen its own 40,000 compound chemical library on two models based on motoneurons: trophic factor dependent survival and a model of excitotoxicity. A specific screen to detect molecules that increase expression of smn-2 is under progress specifically for SMA. One year after completing the primary screen on the trophic factor deprivation model of motoneuron cell death, we have validated our most active lead molecule, TRO19622, in several animal models of motor nerve degeneration and have begun the preclinical development process in order to start Phase 1 studies by the end of this year. We are establishing a clinical development plan to test this molecule in patients with ALS and SMA.

Although TROPHOS' screening is "target-independent", the "validated hits" coming from our chemical collection are using a "reverse engineering" process to identify the molecular targets and mechanisms of action. TRO19622 has an interesting pharmacological profile: by modulating the opening of the mitochondrial pore, it preserves essential mitochondrial functions such as calcium buffering and reduces neuronal death.

We are now developing second-generation assays focused on these new and validated mechanisms that favor neuronal survival. Meanwhile, active compounds are advancing toward the clinic with the skill of heroic chemists willing to formulate structure-activity relationships without a fixed target: the way most drugs came to market before the development of molecular genetic alternatives.
**Background**

Signs of motor neuron degeneration and ubiquitin positive inclusions (UPIs) in the primary motor cortex and at the spinal anterior horn cell level are the hallmarks of amyotrophic lateral sclerosis. However, their abundance varies between cases and the relationship of upper and lower motor neuron degeneration in ALS is unclear. Cases with marked asymmetry may help clarify this issue.

**Objectives**

To determine the histopathological substrate of the clinically observed motor asymmetry.

**Case report**

This 72 year old man whose sister had died of ALS developed slowly progressive clumsiness of his right hand which was followed by weakness of his right leg. He subsequently developed dysarthria and dysphagia. On examination his right arm was plegic and severely atrophied. On the left mild wasting and weakness were restricted to small hand muscles. There were no signs of dementia. One week before the patient died of pneumonia he was still able to stand on his left leg and hold on to a handle bar with his left hand whilst his right side was completely atrophied and plegic. SOD testing was negative.

**Autopsy findings**

Histopathological examination revealed loss of spinal and brain stem motor neurons with UPIs in remaining motor neurons and in granule cells of the dentate gyrus compatible with the diagnosis of ALS. There was severe right-sided predominant axonal degeneration in nerve trunks of the brachial plexus and in lumbosacral anterior spinal roots. The lateral corticospinal tracts showed severe losses of thick axons, more pronounced on the right side. The most striking finding consisted of a severe degeneration of the left precentral motor cortex with neuronal loss, spongiosis, astrocytic gliosis and UPIs in neurons. Only slight changes were observed in the contralateral motor cortex. The prefrontal and temporal cortex only disclosed few ubiquitin-positive neurits.

**Discussion**

The most prominent feature in this patient is marked asymmetry of weakness and wasting affecting mostly his right side, along with neurodegenerative changes of the type found in...
frontotemporal degeneration but restricted to the left motor cortex. There was clear asymmetry in corticospinal tract degeneration probably related to the unilateral lesion of the precentral cortex. Although anterior horn cell degeneration was not found to be asymmetrical by simple inspection, clinical evidence together with clear asymmetry of brachial plexus and lumbosacral root degeneration suggest more severe involvement of anterior horn cells ipsilateral to the most severely affected lateral corticospinal tract.

Our findings suggest that upper and lower motor neurons do not degenerate independently from each other.

**P185 AUTONOMIC DYSFUNCTION OF THE GASTROINTESTINAL SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS: A CASE STUDY**

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**Background**

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder involving principally the motor neurons. Although the El Escorial World Federation of Neurology Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis refers to autonomic dysfunction as an “inconsistent” finding, recent research has shown that in a subset of patients there is involvement of the autonomic nervous system, both clinically and pathologically. Abnormalities have been noted in the circulatory and gastrointestinal (GI) systems as well as in the skin. Pathological basis for these observations has been reported for both skin and the circulatory system, however limited autopsy data is available to support the gastrointestinal (GI) system involvement.

**Objective**

To define the GI tract pathology in a patient with ALS and autonomic dysfunction.

**Methods**

The clinical history and autopsy results of a patient with ALS and autonomic dysfunction were reviewed to correlate pathologic abnormalities with the clinical symptoms.

**Results**

RL was diagnosed with ALS at age 60 when he presented with left lower extremity weakness. He was wheelchair-bound within three years and required mechanical ventilation by 7 years into the course of the illness. Following ventilation he developed symptoms of autonomic dysfunction. He initially had uncontrolled bowel movements, two to three times per day, followed by constipation. Subsequently, he developed lower extremity edema, mottling and coolness, urinary retention, and early satiety. He also had evidence of circulatory collapse, defined as an abnormal fall in blood pressure lower than 80 mm Hg without compensatory tachycardia. He died eight years after the onset of disease.
His autopsy findings were typical for ALS with marked pyramidal tract degeneration, severe chronic anterior horn cell dropout, and moderate degeneration of the substantia nigra and locus ceruleus. In addition, he had moderate neuronal cell loss and of the interomediolateral cell columns and moderate reduction in the number of ganglion cells in the GI tract.

**Discussion**

This patient had symptoms of autonomic dysfunction as well as pathologic findings that correlate with his complaints. His symptoms of circulatory collapse are similar to those previously reported and attributed to sympathetic hyperactivity. While the interomediolateral cell column loss has been reported, the degree of cell loss did not reach statistical significance. While clinical features of esophageal and colonic dysmotility in ALS have been described, there are no pathologic descriptions in these patients. The autopsy of our patient reveals reduced numbers of ganglion cells in the GI tract. Further studies of autopsied ALS patients with and without autonomic dysfunction should be performed to determine the frequency and statistical significance of these findings in the autonomic nervous system and correlate abnormalities to clinical symptoms.

**P186 THE NATIONAL REGISTRY OF US VETERANS WITH ALS**

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In response to concern about the development of ALS in veterans of the United States armed forces, particularly in Gulf War veterans, the Department of Veterans Affairs established a national Registry of veterans with ALS. The goal of the Registry is to identify living veterans with ALS, track the progression of the disease, and determine survival. This will serve to facilitate study of epidemiological risk factors in ALS in the military context and also investigation of gene/environmental risk factors that influence the development and rate of progression.

To date (September 1, 2004), 3,781 potential cases have been identified through active and passive recruitment methods. A total of 432 cases have been reviewed by one of a team of neurologists to validate the diagnosis using the Escorial criteria. Enrolled subjects are followed by phone every six months using the ALSFRS-R.

Supported by the Department of Veterans Affairs Cooperative Studies Program #500a.
P187 BEHAVIOURAL ASSESSMENT OF THE DYSEXECUTIVE SYNDROME: NEUROPSYCHOLOGICAL STUDY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Introduction.
Executive functions permit coping with new and complex situations. The Behavioural Assessment of the Dysexecutive Syndrome, BADS, aimed at predicting every day problems arising from the executive syndrome. There is great variability in the extent and degree of impairment in patients with frontal lobe damage. An executive dysfunction was described in patients with Amyotrophic Lateral Sclerosis (ALS) with frontotemporal dementia (FTD) and in patients with ALS without dementia. The study of the link between ALS and FTD appears to be important at the clinical level.

Methods
We studied neuropsychological performances of 9 patients with ALS associated with FTD, 9 patients with cognitive impairment (but not demented) and 9 ALS patients in comparison to 27 matched controls. The objective was to determinate if some executive performances observed with this new test battery can distinguish the 3 groups of patients.

Results
Considering the total BADS profile score, patients with ALS-FTD performed lower (69.9 ± 4.9) than ALS patients (103.0 ± 9.9) and controls (101.5 ± 13.6) (p < 0.001).

On the 6 executive subtests of the BADS, the Zoo Map test (which requires to plan the visit of a series of designated locations on a map of a zoo) and the Modified Six Elements test (a simplified version of the original Shallice & Burgess, 1991) were likely to show further executive dysfunction in patients with ALS-FTD or ALS patients with mild cognitive impairment compared to ALS patients without cognitive dysfunction or controls.

Conclusion
Assessing everyday problems arising from the dysexecutive syndrome, the BADS was described to be useful for the assessment of executive functioning and provided a tool for picking up subtle difficulties in planning and organisation. Its use appears to be interesting in ALS populations in which frontal/executive dysfunction is currently described.
Background
We have shown that linear estimates of disease progression are predictors of survival in patients with ALS\textsuperscript{1,2}. In anticipation of additional research, which might allow such estimates to be applied to provide individualized prognostic information to patients, we set out to study the degree of interest by patients and relatives in receiving such information and the best time to provide it. As a first step, we polled openly the 31 individuals attending a support group meeting, of whom 15 were patients. Twelve said they would want individualized prognostic information, one did not, and eighteen (58\%) abstained. Regardless of their previous vote, 21 said they would want this information early, i.e., at or as close as possible to the time of diagnosis, and 6 would want the information at a later time; 4 did not participate in the second vote. There was no difference in the responses between patients and non-patients.

Objectives
To follow up on the results of the open poll by conducting an anonymous survey of patients and significant relatives on our mailing list.

Methods
Two questionnaires were sent to the 112 addresses on the mailing list of our newsletter, anticipating one to be for patient and one for caregiver or significant relative. An additional mailing was sent to addresses from which no responses were received within two weeks.

Results
Responses were received from a total of 56 addresses (50\% of 112); of these, 13 were empty responses or indicated unwillingness to participate, for a net response rate of 43 of 112 addresses (38\%). The number of evaluable responses was 54, of which 22 were from patients. There were no major differences between the responses of the patients and of the non-patients: 87\% wanted more precise prognostic information, and 65\% wanted it at or around the time of diagnosis. No patients or spouses wished information to be withheld from the patient, and given to someone else. Three of 11 individuals with other relationships to the patients did have such a preference.

Discussion
The results of this anonymous survey were similar to those obtained in the open poll, with a majority of individuals (about 60\%) choosing not to respond, or abstain, when this question was put to them. This suggests that the availability of more precise prognostic information may not be welcome immediately to all patients and families affected by ALS. Recognition of these patient
preferences may guide how more precise prognostic information is presented to patients, if it becomes available.

References

P189 RESPIRATORY MANAGEMENT OF PATIENTS WITH MND / ALS, AN MND CARE CENTRE EXPERIENCE

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Background
The management of respiratory symptoms in patients with MND/ALS is controversial, with increasing attention to issues including patient assessment, support, and quality of life. We have favoured a practical and pragmatic approach, with a philosophy of symptomatic management, with limited presymptomatic investigation. We describe our experience over the past 2 years.

Objectives
To determine the number of patients assessed, the pattern of assessment, and outcome of patients with MND/ALS and respiratory symptoms, in the past two years, and to determine if any lessons or change in practice are indicated.

Methods
The records of all patients investigated for respiratory symptoms at the MND Care Centre, were reviewed, from September 2001 to August 2003. Ventilatory insufficiency was defined as the development of orthopnoea, increasing breathlessness on exertion, or breathlessness at rest, taken together with vital capacity <1 litre, and evidence of diaphragmatic paradox, or nocturnal hypoventilation.

Results
246 patients were seen, 98 new and 148 follow up. These represented 126 different patients. Two patients were intubated and ventilated elsewhere as the result of acute ventilatory failure. Noninvasive ventilation (NIV) with NIPPY was successfully instituted in 7 patients (1 with tracheostomy, the others with a mask), and not tolerated in a further 3 patients. 25 patients were admitted for sleep studies, with a total of 39 sleep studies performed, on the basis of symptoms or reduced vital capacity. One patient had elective tracheostomy with an uncuffed tube. Two patients
had emergency tracheostomy for episodes of respiratory failure precipitated by bronchopneumonia.

**Discussion and conclusions**
We offered respiratory support to patients with MND/ALS. The proportion of patients requiring continuing support was relatively low. We did not institute respiratory support in patients with problems including insufficient home support, progressive bulbar weakness, or severe accompanying limb weakness, and the primary determinant was patient choice. It was apparent that there was a change in patients early expressed wishes as the disease progressed. This pattern of assessment and management appears to work well in the patient population and resource environment in which we practice.

**P190 “HOW TO COMMUNICATE IF ONE OF THE PARTNERS CAN NOT SPEAK”**

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At this presentation delegates will be given the opportunity to try the interactive sessions in the online e-learning program: “How to express yourself when speech is lost”.

Further information is given in the abstract (P191) below.

**P191 “HOW TO EXPRESS YOURSELF WHEN SPEECH IS LOST”**

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This presentation will be a short demonstration of our online data-program: “How to express yourself when speech is lost”.

“How to express yourself when speech is lost” is an e-learning program developed at the Centre for Assistive Information technology at the Norwegian National Insurance Administration in co-operation with speech and language pathologist from Sunnaas University Rehabilitation Hospital, Norway. In the years 2001 – 2003 she travelled round Norway, making interviews with users of AAC devices and their families. The project was funded by the Norwegian Foundation for Health and Rehabilitation.
The objects of this program are 1) to illustrate that a means of communication through AAC devices exists when language is still present, but speech has been lost and 2) to give anyone the possibility to learn about views and knowledge of users and professionals on AAC.

The content includes: videoclips showing users of AAC devices; short articles about AAC methods and devices; demos of some of the devices with an opportunity to try some of them (eye pointing and on screen keyboard with scanning) and the possibility to reflect over issues given by the program

We hope that this program will give AAC users, their families and professionals more knowledge in this field and thereby a greater possibility to contribute with wishes and demands in improving their own position.

Who is it for?
People who have or may develop a speech impairments, for their families, friends and professionals caring for them.

Website: [http://www.trygdeetaten.no/ikthjelpemidler](http://www.trygdeetaten.no/ikthjelpemidler)
In Denmark there are 2 centres for home mechanical ventilation, who provides the set-up and training program for home mechanical ventilation treatment. Our centre has 47 patients with ALS connected. One patient does not have any respiratory support. Seven patients use C-pap and 22 patients live with a Bi-pap. 17 are tracheostomised and connected to invasive mechanical ventilation. They all live at home with a personal helper team.

Background
The employer is the patient. The municipality subsidizes the cost to employ a care team for people with a permanent and serious disability, who live an active life, and require special support.

If a patient does not have the ability to be an employer, the municipality will take over and employ the care team. The municipality is obligated to offer personal aid, care and practical support at home. The support will be offered to people with temporary or permanent physical or psychological disability or people with serious social problems.

The personal helper teams are ordinary people, where no care qualifications are required. The Centre for Home Mechanical Ventilation provides a 3-4 weeks training program for the patient and the helper team.

The social and financial details of this programme are as follows: the patient has their pension (disabled). The cost to employ the care team is paid by the municipality. Gloves, catheters for suction, tissues etc. are delivered by the municipality.

Conclusion
To get home mechanical ventilation and the possibility of a good quality of life does not depend on your finances. With your personal helper team you can live an active live with social activities, journeys etc. without depending on your family. The personal helper does all the personal care, allowing the patient to live at home.
Introduction
ABRELA is a Brazilian civil entity, with no profitable returns, that has as its objectives: 1. to increase the awareness of the Brazilian society about the importance and the emergence of amyotrophic lateral sclerosis (ALS), by means of epidemiological studies, scientific meetings, and regular delivery of information to health professionals; 2. to promote a better quality of life for ALS patients and their families, through information, orientation and social support. ABRELA was founded in 1998 by neurologists interested in the illness and its treatment, in view of the difficulties of ALS patients and their families in dealing with the disease. The social work of ABRELA initiated its work in 2000; since then it has developed actions to reach the objectives of ABRELA, by providing the social attendance and setting up a fundraising committee, as well as promoting partnerships with other public and private institutions.

Objectives
The objective of this report is to describe the activities of the social work of ABRELA during the last four year period (2000-2003).

Material and methods
All of the reports of face-to-face and telephone interviews made by the social service of ABRELA during the period of 2000-2003 were revised and information concerning epidemiological and social data was collected. Moreover, the register of social, scientific and academic activities of ABRELA was researched.

Results
In the last four years period (2000 to 2003) the social service of ABRELA made 7,125 personal or telephonic interviews, a mean of 1781 interviews per year. These interviews referred to 1,023 ALS patients, 3,756 ALS relatives, 1,960 health professionals and 386 people interested in learning about ALS/ABRELA. In the group of ALS patients, age ranged from 20 to 80 years old and there were 344 women and 472 men. Patients came from all the regions of Brazil, but the great majority (80%) was from the southeast of the country. Requests were related to information about 1. medicines, 2. public health assistance, 3. ventilatory assistance, 4. home care services, 5. taxes, and 6. social security. Since 2000, ABRELA has held bimonthly social / scientific meetings for patients and relatives, and biannual scientific meetings for health professionals. Moreover, ABRELA efforts have obtained the official regulation of the free distribution of Riluzole, percutaneous endoscopic supplies and noninvasive ventilation devices as BIBAP. Finally, ABRELA has accomplished partnerships for outpatient multidisciplinary clinic attendance (Federal...
University of São Paulo); psychological domiciliary attendance (University of São Paulo) and tutor domiciliary visits (Psychology and Nursing graduate students).

Conclusions
The activities of ABRELA have increased the social awareness about ALS and the availability of the means for increasing the quality of life of patients and their relatives. Unfortunately, it seems that the progress is still restricted to the region near ABRELA, in the southeast of Brazil. Efforts must be directed to expand our action to all the country.

P194 BREAST CANCER IN WOMEN WITH MOTOR NEURON DISEASE

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Background
A few cases of breast cancer (BC) associated with amyotrophic lateral sclerosis (ALS), especially primary lateral sclerosis (PLS) have been reported\(^1,2\) but the relevance of these facts is still poorly understood.

Objectives
To report 6 additional cases of ALS associated with BC.

Methods
We retrospectively searched for any history or present occurrence of BC in women presently attending our ALS consultation.
Results
The results are summarized in the table. Six women with ALS, aged 52-81 years (mean: 68.5) had present or past BC. The mean duration of ALS was 6.2 years. Three had PLS (one with associated fronto-temporal dementia), and in the remaining 3 the lower motor neuron involvement was clinically significant in one woman only. The BC antedated the ALS by a mean of 5 years in 5/6 cases and followed it by 6 years in the remaining one.

<table>
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<th>N°</th>
<th>Age at the time of study</th>
<th>ALS duration /onset</th>
<th>Anti-neuron antibodies</th>
<th>EMG</th>
<th>Cancer onset (years before MND)</th>
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<tr>
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<td>2/spinal</td>
<td>Negative</td>
<td>Normal</td>
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</tr>
<tr>
<td>2</td>
<td>78</td>
<td>6/spinal</td>
<td>Not done</td>
<td>Normal</td>
<td>2*</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>2/spinal</td>
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<td>Normal</td>
<td>32</td>
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<td>4</td>
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<tr>
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<td>81</td>
<td>4/spinal</td>
<td>Pending</td>
<td>Neurogenic</td>
<td>25</td>
</tr>
</tbody>
</table>

*associated colonic cancer

Discussion
Our data confirm that, among women with ALS, a history of BC is mostly found in patients with PLS. No antibodies have been found either in the present or in previous studies. However, this association seems not to be coincidental, in view of the low prevalence of PLS among the whole MND complex.

Conclusion
The relationship of BC and PLS deserves further consideration and research. BC should be specifically looked for in women with PLS.

References