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SESSION 1 JOINT OPENING SESSION

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

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Keywords: genetics, epidemiology, risk factors

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

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

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

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

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SESSION 2A RNA & PROTEIN DYSREGULATION

C2 CELLULAR IMPLICATIONS OF RNA REPEATS IN MYOTONIC DYSTROPHY

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Keywords: RNA repeats, myotonic dystrophy, RNA mechanism

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

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

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Keywords: RNA metabolism, RGNEF, p62

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

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

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

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

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

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

References

1. Strong MJ. J Neurol Sci 2010;288(1–2):1–12.
2. Keller BA, Volkening, K, Droppelmann, CA *et al.* Acta Neuropath, Submitted.
3. Droppelmann CA, Keller BA, Campos-Melo D *et al.* Neurobiol Aging, Submitted.
4. Zielinski J, Kilk K, Peritz T *et al.* Proc Natl Acad Sci USA 2006;103:1557–1562.

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

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

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

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

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

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

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

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

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Keywords: FUS, postranslational modification, ALS pathogenesis

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

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

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

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

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

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

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

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Keywords: FUS, transportin, arginine methylation

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

References

1. Dormann D, Haass C. Trends in Neuroscience 2011;7: 339–348.
2. Dormann D, Rodde R, Edbauer D *et al.* EMBO J. 2010; 29:2841–2857.

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C7 DYSREGULATED MICRORNAS IN THE PATHOGENESIS OF ALS

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Keywords: microRNA, RNA, regulation

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

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

Methods and results: We quantified microRNA expression in samples extracted from laser-capture microdissected spinal motoneuron punches of ALS patients who had met El Escorial criteria for definite ALS and of controls that were not reported to suffer from neurodegeneration. Tissue collections were completed within 4–6 h of death and RNA quality was assessed using microelectrophoresis on an Agilent 2100(1). Quantification of 667 microRNAs performed by using microRNA TaqMan® qPCR Megaplex pool arrays. This study revealed global downregulation of microRNAs in ALS lower-motoneurons from the lumbar region of sporadic and familial cases, but not in RNA extracted from surrounding, neuron-depleted ventral horn tissue or from the neurons of Clarke's column in the same autopsies. This observation was substantiated by in-situ hybridization, which revealed comparable downregulation of microRNAs in patient tissue, relative to control.

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

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

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

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

References

1. Rabin SJ *et al.* Hum Mol Genet 2010;19:313–28.
2. Emde *et al.*, unpublished results.
3. Haramati S *et al.* Proc Natl Acad Sci USA 2010;107: 13111–6.

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C8 MUTATIONS IN PROFILIN 1 CAUSE FAMILIAL ALS

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Keywords: exome sequencing, mutations, insoluble aggregates

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

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SESSION 2B COGNITIVE CHANGE

C9 ALS/FTD: CORRELATIONS WITH PATHOLOGY

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

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

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Keywords: cognition, subphenotypes, frontotemporal dementia

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

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

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

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

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

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

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

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Keywords: FTD, gender, cognition

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

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

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

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

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

References

1. Johnson JK, Diehl J, Mendez MF, *et al.* Arch. Neurol. 2005;62(6):925–30.
2. Kaiser A, Kuenzil E, Zappatore D, Nitsch C. Int. J. Psychophysiology 2007;63:192–198.
3. Minzenberg MJ, Poole JH, Benton C, Vinogradov S. Am. J. Psychiatry 2004;161(1):116–124.
4. Riccio CA, Hynd GW. J. Psychoeducational Assess 1992; 10(3):210–218.
5. Callender TJ, Morrow L, Subramanian K, Duhon D, Ristovv M. Environ. Res. 1993;60:295–319.

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

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Keywords: disease progression, ALSFTD, ALSFRS-R

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

References

1. Strong MJ, Grace GM, Freedman M *et al.* ALS 2009;10: 131–46.
2. Phukan J, Elamin M, Bede P *et al.* JNNP 2012;83: 102–108.
3. Rascovsky K, Hodges JR, Knopman D *et al.* Brain 2011; 134:2456–77.
4. Sarro L, Agosta F, Canu E. AJNR 2011;32:1866–72.

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SESSION 3A CELL STRESS MECHANISMS

C15 SELECTIVE AUTOPHAGIC DEGRADATION OF AGGREGATE-PRONE PROTEINS

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

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

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

References

1. Clausen TH, Lamark T, Isakson P. *et al.* Autophagy. 2010;6(3):330–44. Epub 2010/02/20.
2. Filimonenko M, Isakson P, Finley KD. *et al.* Alfy. Mol Cell. 2010;38(2):265–79. Epub 2010/04/27.
3. Filimonenko M, Stuffers S, Raiborg C, *et al.* Journal of Cell Biology. 2007;179(3):485–500.

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

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Keywords: TDP43, FUS, ER-Golgi transport

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

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

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

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

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

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

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

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Keywords: autophagy, aggregates, SOD1 mutant

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

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

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

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

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

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

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Keywords: FTD, dementia, UBQLN2

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

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

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

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

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

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

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C19 PROPAGATED MISFOLDING OF SOD1 IN FAMILIAL AND SPORADIC ALS

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

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

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

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

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

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SESSION 3B AUTONOMY AND DECISION MAKING

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

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

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

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

Reference

1. Emanuel E, Emanuel L. Four models of the physician-patient relationship. JAMA 1992;267:2221–2226.

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

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Keywords: death, suicide, end-of-life

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

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

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

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

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

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

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

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

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

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Keywords: quality of life, depression, decisions

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

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

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

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

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

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

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

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

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

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

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

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

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C24 ALS PATIENT REPORTS OF END OF LIFE PREFERENCES

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

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

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

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

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

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

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SESSION 4A GENETICS & GENOMICS

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

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Keywords: multisystem proteinopathy, inclusion body myopathy, frontotemporal dementia

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

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

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

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

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

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

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Keywords: C9ORF72, southern blot, repeat length

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

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

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

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

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

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

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C27 EVIDENCE FOR AN OLIGOGENIC BASIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oligogenetic inheritance, C9ORF72, TARDBP

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

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

Results: Mutations were identified in 48% of FALS families, 8% of SALS patients, and 0.5% of control subjects. In five of the FALS families, we identified multiple mutations in multiple ALS-associated genes. We detected *FUS/TLS* and *TARDBP* mutations in combination with *ANG* mutations, and *C9ORF72* repeat expansions with *TARDBP*, *SOD1*, and *FUS/TLS* mutations. Statistical analysis demonstrated that the presence of multiple mutations in FALS is in excess of what is to be expected by chance ($p=1.77\times10^{-7}$). The most compelling evidence for an oligogenic basis was found in individuals with a p.N352S mutation in *TARDBP*, detected in five FALS families and three apparently SALS patients. Genealogical and haplotype analyses revealed that these individuals shared a common ancestor. We obtained DNA of 14 patients with this *TARDBP* mutation, 50% of whom had an additional mutation (*ANG*, *C9ORF72* or homozygous *TARDBP*).

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

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

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Keywords: exome, linkage, bioinformatics

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

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

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

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

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

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

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

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Keywords: genetics, 9p21, statistics

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

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

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

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

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

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

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

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Keywords: C9ORF72, RNA sequencing, expression

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

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

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

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

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

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SESSION 4B CARER & FAMILY SUPPORT

C31 HOW CAN WE CARE FOR THE CARERS?

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Keywords: carer, stress, loss

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

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

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

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

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

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

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C32 LIVING WITH MND: A CARER PERSPECTIVE

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Keywords: carer burden, qualitative, quality of life

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

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

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

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

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

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

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

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Keywords: purpose in life, interpersonal relationships, survival

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

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

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

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

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

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

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

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

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

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

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

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

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

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C35 SPECIFIC PHOBIA OF AMYOTROPHIC LATERAL SCLEROSIS (ALS PHOBIA)

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Keywords: specific phobia, psychotherapy, affective disorders

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

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

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

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

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

References

1. Krasnov VN. Affective disorders. Moscow, Practical Medicine 2011;431 (in Russian)
2. Narrow WE, Rae DS, Robins LN. *et al.* Ach. Gen. Psychiatry 2002;2:115–23.
3. Wittchen HU, Jacobi F. Eur Neuropsychopharmacol 2005; 15:357–76.

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SESSION 7A MODELLING ALS

C36 MODELLING ALS WITH EMBRYONIC STEM CELL-DERIVED MOTOR NEURONS

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

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

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

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Keywords: embryonic stem cells, TDP43, motor neurons

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

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

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

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

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

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

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C38 ZEBRAFISH TO IDENTIFY MODIFIERS OF ALS

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Keywords: zebrafish, EphA4

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

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C39 LOSS OF TDP-43 CAUSES HYPOPERFUSION AND MUSCLE DYSTROPHY

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Keywords: TDP-43, mutant, zebrafish

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

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

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

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Keywords: TDP-43, chemical genetics, animal models

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

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

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

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

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

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

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

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

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Keywords: FUS, Drosophila, locomotion

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

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

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Keywords: FUS/TLS, aggregation, transgenic mice

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

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

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

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

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

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SESSION 7B CLINICAL TRIALS & TRIAL DESIGN

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

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Keywords: antisense, SOD1, phase I

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

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

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

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

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

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

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Keywords: stem cells, transplant, spinal cord

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

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

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

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Keywords: clinical trial, muscle activator, pharmacokinetics

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

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

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

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

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

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

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Keywords: olesoxime, clinical trial, phase 3

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

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

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

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

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

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

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Keywords: biomarkers, clinical trial, treatment

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

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

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

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

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

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Keywords: ALSFRSr, abbreviated ALSFRS-6

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

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

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

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

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

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

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C49 A HEALTH STATE STAGING SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: health states, staging system, delphi process

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

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

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

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

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

Reference

1. Roche *et al.* Brain 2012;135:847–852.

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SESSION 8A CELLULAR DIVERSITY AND SELECTIVE VULNERABILITY

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

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Keywords: corticospinal, upper motor neurons

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

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

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

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

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

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

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Keywords: AAV, corticospinal motor neuron degeneration, neuroinflammation

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

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

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

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

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

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

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

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

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

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

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

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

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

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

References

1. Song F, Chiang P, Wang J, Ravits J, Loeb JA. Aberrant J Neuropathol Exp Neurol 2012;71:104–15.
2. Ravits J, Laurie P, Fan Y, Moore DH. Neurology 2007;68: 1576–82.

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

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Keywords: neurotrophic factors, motor neuron subsets

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

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

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

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

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

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Keywords: Selective vulnerability, oculomotor neuron, excitotoxicity

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

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

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

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

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

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

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SESSION 8B CLINICAL REGISTERS AND EPIDEMIOLOGY

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

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Keywords: population-based, registers, registries

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

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

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

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

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Keywords: epidemiology, database, survival

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

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

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

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

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

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

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

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

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Keywords: comorbidities, venous thromboembolism, pulmonary embolism

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

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

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

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

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

References

1. Chio A, Canosa A, Cammarosano S *et al.* Neurology 2011; 77:1432–7.
2. Qureshi MM, Cudkowicz ME, Zhang H *et al.* Neurology 2007;68:76–7.
3. Elman LB, Siderowf A, Houseman G *et al.* Amyotroph Lateral Scler 2005;6:246–9.
4. Sorenson EJ, Windbank AJ, Mandrekar JN *et al.* Neurology 2008;71:1770–1775.

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C58 MEDITERRANEAN DIET MODIFIES RISK OF AMYOTROPHIC LATERAL SCLEROSIS

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

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

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

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

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

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

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

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C59 SEVERE VITAMIN D DEFICIENCY CORRELATES WITH WORSE ALS PROGNOSIS

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Keywords: vitamin D, prognosis, neuroprotection

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

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

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

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

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

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SESSION 9A PHENOTYPIC CHANGE/ MODIFICATION

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

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Keywords: SOD1, gross phenotype, harmful effect

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

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

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

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Keywords: gene expression profile, laser captured motoneurons, neuroimmunity

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

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

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

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

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

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

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Keywords: PGC-1a, metabolism, genetic modifier

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

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

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

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

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

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

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Keywords: PGC1a, muscle, therapy

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

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

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

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

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

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

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

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

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

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Reference

1. Ludolph *et al*. ALS 2010.

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

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

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

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

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

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

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

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

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SESSION 9B MULTIDISCIPLINARY MANAGEMENT

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

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Keywords: quality improvement, benchmarking, clinical audit

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

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

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

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

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

References

1. Williams NM, Bravver EK, Bockenek WL, *et al.* ALS 2011; 2(Suppl 1):12.
2. American Academy of Neurology 2011; www.aan.com/globals/axon/assets/9281.pdf
3. The Joint Commission 2012; www.jointcommission.org/assets/1/18/Facts_about_Disease_Specific_Care_Certification.pdf
4. The Joint Commission 2012; "Disease-Specific Care Certification" www.jointcommission.org/certification/certification_main.aspx

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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C69 ALSPARTNER - INTERNET PLATFORM FOR COMPREHENSIVE ALS CARE

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Keywords: internet, home care, devices, aids

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

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

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

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

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C70 BULBAR MOTOR DETERIORATION IN ALS

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Keywords: bulbar deterioration, speech production, bulbar assessment

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

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

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

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

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

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

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

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

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Keywords: survival, prognostic factors, therapeutic intervention

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

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

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

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

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SESSION 10A ROLES OF NON-NEURONAL CELLS

C72 NEURON-ASTROCYTE CROSSTALK IN ALS

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

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Keywords: astrocytes, lysosomes, cholesterol

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

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

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

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

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

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Keywords: astrocytes, SOD1 gene therapy

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

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

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

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

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

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

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

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

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

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

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Keywords: TDP43, astrocyte, glia

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

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

Methods: Glial-restricted precursors (GRPs) were isolated from $TDP43^{A315T}$, $SOD1^{G93A}$, or wild-type (WT) littermate mice and differentiated to astrocytes *in vitro* or transplanted to the spinal cord of WT rats for *in vivo* astrocyte differentiation. The effects of the astrocytes on WT MN survival were determined *in vitro* using a co-culture system or *in vivo* by examining host MNs at three months post-transplantation.

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

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

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C76 IMMUNE MODULATION AS A THERAPEUTIC STRATEGY FOR ALS

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Keywords: immune modulation, neuroinflammation, macrophage

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

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

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

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

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

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

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SESSION 10B RESPIRATORY SUPPORT

C77 MULTIDISCIPLINARY RESPIRATORY CARE IN ALS

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Keywords: respiratory, ventilation, pulmonary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Keywords: respiratory failure, TOSCA 500, carbon dioxide levels

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Keywords: diaphragm dysfunction, radiology, diaphragm pacing

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

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

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

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

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

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SESSION 11A MURINE MODELS

C82 WHAT MAKES A GOOD ANIMAL MODEL?

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Keywords: rodent model, knockout mice, TDP-43

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

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C83 NEW ANIMAL MODELS OF ALS AND ALS/DEMENTIA

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Keywords: UBQLN2, OPTN, transgenic

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

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

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

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

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

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Keywords: FUS, transgenic mice, motor impairment

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

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

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

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

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

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

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Keywords: FUS, mouse, model

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

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

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

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C86 DEGENERATION OF MOTONEURONS IN SOD1 MICE: THE SIZE HYPOTHESIS

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Keywords: morphology, 2 photon imaging, excitotoxicity

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

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

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

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

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

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

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

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

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Keywords: excitotoxicity, neuromuscular junction, axonal degeneration

Background: ALS is likely to be a multifactorial disease of neuronal dysfunction and loss, however, recent investigations indicate that axonal dysfunction, prior to cell loss, may be the causative factor of the initial symptoms of ALS and that distal axonal degeneration may occur before the onset of disease symptoms.

Purpose: Our investigations are focused on determining the degenerative changes underlying ALS-like axonopathy by using site-specific excitotoxic insults, via osmotic minipumps, to the spinal cord and muscle.

Methods: To achieve site-specific excitoxotixity osmotic mini pumps (Alzet, model 1004), delivered a constant chronic infusion to either the L3-4 lumbar region of the spinal cord or the gastrocnemius muscle in the hind limb. A constant and chronic infusion of Kanic acid (KA, 1–5mM, in cortex buffer with 1μM Fluro Ruby) was delivered to the subarachnoid space of the lumbar region (L3-5) of C57/Bl6 mice and transgenic mice which express yellow fluorescent protein (YFP) in a subset of motor neurons on a C57/BL6 background. At the gastrocnemius muscle glutamate (5mM in saline with 1μM Fluro Ruby) was chronically infused in YFP mice. Animals were perfused at a range of time points and the degree of axonal degeneration was investigated through immunohistochemistry and confocal microscopy.

Results: Fluoro Ruby labelling was present throughout cells within the subarachnoid space in L3-5 and muscle fibres of the gastrocnemius muscle, indicating a targeted delivery can be achieved with the osmotic pumps. Quantitation of the number of neurons (cell body > 20 μm) stained with toluidine blue within the anterior ventral horn at 7, 14 and 28 days post surgery (DPS) demonstrated a significant ($p < 0.05$) decrease in the number of motor neurons at 28 DPS in the 5 mM KA treated mice in comparison to vehicle control. Gastrocnemius muscles of the KA and vehicle control spinal cord treated mice were double labelled with synaptophysin and alpha-bunglarotoxin to determine the amount of neuromuscular junction (NMJ) degeneration. Synapses were graded as either intact or degenerating. There was a significant ($p < 0.05$) increase in the percentage of degenerating synapses in the KA mice in comparison to control. Additional, in the YFP mice, analysis demonstrated that whilst there was a reduction in the number of synapses in the KA muscle there was also an increase in the number of branch points in each junction, indicating that whilst degeneration of the NMJ was present, NMJ remodelling was also occurring in the KA-treated mice. This compensatory plasticity may be an early event in the pathogenesis of axonal degeneration in ALS.

Conclusion: Identifying the site of the initial effects of excitotoxicity will identify mechanisms of distal axon degeneration that may provide novel therapeutic targets directed at axon protection.

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

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Keywords: neuromuscular junction, microendoscopy, activity

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

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

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SESSION 11B SURROGATE MARKERS

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

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Keywords: PET, C9ORF72, neuroimaging

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

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

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

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

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

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

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Keywords: microglia, DPA714, TEP imaging

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

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

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

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

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

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

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

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Keywords: spinal cord, atrophy measurement, progression biomarker

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

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

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

Results: Wilcoxon signed rank test showed a significant decrease over time in mean cord area (-2.8%, $p = 9x10^{-4}$). Spearman's coefficient showed significant correlation between mean cord sectional area changes and the ALSFRS-R upper limb changes ($r = 0.83$, $p = 2\times10^{-4}$).

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

References

1. Agosta F, Rocca MA, Valsasina P. *et al.* JNNP 2009;80: 53–55.
2. Cohen-Adad J, El-Mendili MM, Pradat PF, *et al.* ALS 2012; minor revision.
3. Cohen-Adad J, El-Mendili MM, Morizot-Koutlidis R, *et al.* Neuroimage 2011;55(3):1024–1033.
4. Lundell H, Barthelemy D, Skimminge A, *et al.* Spinal Cord 2011;49(1):70–5.

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C92 THE EARLIEST CHANGES IN MOTOR UNIT PHYSIOLOGY IN ALS

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Keywords: electromyography, fasciculation potentials, disease progression

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

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

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

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

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

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

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Keywords: MUNIX, ALSFRS-R, biomarker

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

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

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

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

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

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C94 IS SERUM CREATININE A BIOMARKER FOR ALS?

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Keywords: biomarkers, clinical trials, trial design

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

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

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

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

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

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

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

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

Reference

1. Paillisse *et al.* ALS 2005;6:37–44.

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C95 A NOVEL BIOMARKER STRATEGY FOR PREDICTING ALS PROGNOSIS

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Keywords: biomarker, prognosis, predictive model

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

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

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

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

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

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C96 BODY FLUID BIOMARKERS FOR MOTOR NEURON DISEASE

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Keywords: biomarkers, urine, ELISA

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

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

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

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

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

References

1. Bowser R, Turner MR, Shefner J. Nature Reviews Neurology 2011;7:631–638.
2. Seeburger J, Tarras S, Natter H, *et al.* Brain Research 1993;621:111–115.
3. Copray J, Jaarsma D, Ku B *et al.* Neuroscience 2003;116: 685–694.
4. Flinders University, Australia, Biomarker for Motor Neuron Disease 2011. PCT 2011900312.

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