P130 MUTATIONS IN A NEW ALS/FTD GENE, CCNF, UNCOVERS NEW MECHANISMS OF LYS48-UBIQUITYLATION REGULATION BY THE SKP-CUL-F-BOX (CYCLIN F) (SCF (CYCLINF)) E3 LIGASE COMPLEX

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Keywords: proteomics, ubiquitylation, phosphorylation

Background: We recently identified mutations in the CCNF gene as a novel cause of ALS/FTD (1), with the p.Ser621Gly mutation found to segregate across multiple generations in an Australian family. CCNF encodes cyclin F, an E3 ubiquitin ligase that forms a part of a SCF complex that binds to proteins for ubiquitylation and degradation by the UPS. Experimental expression of the CCNF (p.Ser621Gly) mutant protein led to defective protein degradation and features of ALS pathogenesis in vitro (1). We investigated the effect of the CCNF p.Ser621Gly mutation on Lys48-specific ubiquitylation of substrates, and how this mutation alters its E3 ligase activity and stability that contributes to the ubiquitylation of neuronal proteins. We examined the phosphorylation status of cyclin F at Serine621 and how this site regulates the Lys48-specific ubiquitylation activity of the SCF (Cyclin F) complex.

Methods: Neuro2A or HEK293 cells were transfected with either mutant cyclin F (p.Ser621Gly) or wild-type cyclin F fused with N-terminal mCherry. Lys48-ubiquitylated proteins were immunoprecipitated from cell lysates (n=5), digested with trypsin and identified by LC-MS/MS with stringent filtering criteria. In a parallel study, phosphorylation sites were identified by immunoprecipitating mCherry-cyclin F, digestion with trypsin or Asp-N, TiO2 enrichment of phosphopeptides, followed by LC-MS/MS. E3 ligase activity of the SCF(Cyclin F) complex was evaluated by an in vitro ubiquitylation ELISA activity assay.

Results/Discussion: Mass spectrometric analyses identified seven phosphorylation sites (five unique including Serine621) on cyclin F. Phosphorylation at Serine621 in wild-type cyclin F reduced the Lys48-ubiquitylation activity of SCF(Cyclin F) by ~1.35-fold (n=3, p<0.01) compared to the cyclin F(p.Ser621Gly) mutant. Due to the differences in E3 ligase activity, RRM2 (a known cyclin F substrate) expression was elevated by ~1.3-fold (n=3, p<0.05) and ubiquitylated mostly with Lys48-ubiquitin in mutant cyclin F(p.Ser621Gly) transfected cells while RRM2 in the wild-type cyclin F control lysates was ubiquitylated with both Lys48- and Lys63-ubiquitin. LC-MS/MS identified 395 and 205 Lys48-immunoprecipitated proteins from wild-type cyclin F and cyclin F(p.Ser621Gly) transfected cells, respectively. Of these, Gene Ontology and IPA analysis identified a higher percentage of Lys48-ubiquitylated proteins in cyclin F(p.Ser621Gly) cells clustered into biological networks that are responsible for cellular survival and maintenance. The Serine621 phosphorylation site is a key regulator of E3 ligase activity, with the glycine mutation preventing phosphorylation and elevating Lys48-ubiquitination activity. We show differences in wild-type cyclin F and cyclin F(p.Ser621Gly) Lys48-ubiquitylated proteins with the mutant form targeting a larger proportion of substrates linked to cell survival. These findings highlight new mechanisms of cyclin F phosphorylation and its convergence to ubiquitin-mediated regulation of substrates responsible for sustaining the cellular milieu.

Acknowledgements: This research was supported by grants from MNDRIA (GIA1628); and NHMRC (APP1107644 and APP1095215), and access to APAF established under the Australian Government's NCRIS program.

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DOI: 10.1080/21678421.2016.1232060/001

P131 INVESTIGATING THE ROLE OF CYCLIN F IN ALS DISEASE PATHOLOGY

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Keywords: BioID, affinity-capture, proteomics

Background: Our team has identified novel missense mutations in CCNF, some of which lead to aggressive ALS pathology. At the protein level, cyclin F is a component of an E3-ubiquitin ligase which has only four known substrates - all linked to cell cycle progression. This makes it difficult to link cyclin F mutations with disease initiation and progression. A challenge when identifying substrates of E3 ligases is transient binding between the ligase and its substrates. This aspect of the ligase-substrate interaction will be addressed in this study.

Objectives: The first objective is to identify novel substrates of cyclin F using proximity-based biotinylation
(BioID) and mass spectrometry (MS). The second objective is to validate high confidence interactions using immunoprecipitations (IPs) and MS.

**Method:** BioID is a new methodology that involves fusing mutant biotin ligase in frame with the enzyme of interest (cyclin F) in order to biotinylate proteins that come into close proximity. We generated a monoclonal, stably transfected, tetracycline-inducible HEK293 cell line that expresses cyclin F-BioID fusion protein. Stable HEK293 cells expressing cyclin F-BioID were selected for using hygromycin and blasticidin. After tetracycline-induced protein expression, cells were treated with 10 μM MG132 for 24 h alongside 5 μM biotin for 24 h to biotinylate proteins that are within close proximity to the cyclin F-BioID fusion protein. After cell lysis, biotinylated proteins were enriched by streptavidin-bead pull downs. Biotinylated proteins were digested in-solution with trypsin and analyzed by LC-MS/MS. In addition to this, standard IP of Cyclin F, followed by in-solution trypsin digestion was used as a complementary approach to identify interacting partners of cyclin F.

**Results:** We identified 197 proximal proteins by LC-MS/MS. DAVID and IPA bioinformatic analysis revealed these potential interacting partners that clustered into networks that had roles in several biological processes including protein refolding and synthesis, cell growth and proliferation, and RNA-binding and processing. One of these potential binding partners has thus far been validated using IP-MS and immunoblotting.

**Discussion and conclusions:** Our preliminary results indicate that cyclin F interacts with collective subsets of proteins that are responsible for various cellular processes including protein refolding and RNA metabolism, which contributes to maintaining homeostasis. Cyclin F has well characterized mechanisms in cell cycle progression, however, given the limited number of substrates, it is likely that the role of cyclin F in other cellular processes is largely unknown. BioID followed by MS is the first step to expanding the roles Cyclin F plays in the cell, which may aid in linking mutations in cyclin F to altered signaling pathways and ultimately neurodegeneration.

**Reference**

1. Williams KL, et al., Nat Commun. 2016;7:11253.

DOI: 10.1080/21678421.2016.1232060/002

**P132 CHARACTERIZATION OF NEURONAL TOXICITY IN AMYOTROPHIC LATERAL SCLEROSIS**

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**Keywords:** ipsc, motorneuron, astrocyte

**Background:** Amyotrophic lateral sclerosis (ALS) is a devastating progressive neurodegenerative disease characterized by the loss of motor neurons, resulting in muscle weakness and paralysis. The most commonly observed ALS causative genes are SOD1, FUS, TARDBP, and C9ORF72. For ALS studies it is noteworthy that relevant tissues, and particularly live CNS cells from affected individuals, are not readily obtainable. Recent advances in stem cells biology have provided exciting opportunities to develop disease-specific cell types that allow us to understand and explore mechanisms that contribute to pathogenesis of disease. In particular, the ability to reprogram somatic cells into induced pluripotent stem cells (iPSC) provide obvious advantages to generate patient-specific iPSC that carry the exact same genetic makeup, including mutations that may contribute to disease process.

**Objectives:** The disease relevant cell types will be ALS patients' motor neurons (MN) and astrocytes that are differentiated from human-induced pluripotent stem cells (hiPSC) derived from primary fibroblast cultures established from skin biopsies. Our three ALS related assays will monitor the toxicity of patient derived MN when exposed to: (1) supernatant from astrocyte cultures derived from ALS mouse models; and (2) organophosphates (Ops). Using multiple disease relevant assays and cells from different patients ensures that we will test many of the important therapeutic mechanisms and pathways in ALS.

**Methods:** Fibroblasts will be prepared from skin biopsies of consenting ALS patients recruited for this study. The fibroblasts will then be used to generate two lines of hiPSCs per patient according to established protocols. A Brain Canada platform makes our hiPSCs, genome-edits them from fibroblasts and assists in the differentiation into different neural cell types. The Laval hub of this platform (directed by Dr Fon) derives and reprograms patients’ fibroblasts into hiPSC and the Montreal hub of the platform (directed by Dr Fon) differentiates them and genome edits them using CRISPR/Cas9.

**Results:** Fibroblasts from 6 C9ORF72, 1 FUS, and 1 SOD1 case and 2 control individuals have already been collected and are ready for the preparation of hiPSC. A three-step strategy is used to induce robust MN differentiation from hiPSCs. The motor neurons’ nature was confirmed by testing for characteristic markers (e.g. HB9, ChAT, Tuj1). Astrocytic differentiation is now routinely done by treating hiPSCs with FGF2 and EGF. The astrocytic nature of these hiPSC-derived cells will be confirmed by GFAP immunocytochemistry and a panel of additional markers (BDNF, GDNF).

**Discussion and conclusions:** hiPSC-derived MN and astrocyte cultures from our deeply phenotyped ALS cases are the ideal model to develop disease relevant assays. These assays can be used to test for molecules that can alter MN cell survival/reduce the presence of pathogenic protein aggregates and/or RNA foci in those MN.

DOI: 10.1080/21678421.2016.1232060/003
CRISPR/CAS9-MEDIATED GENE CORRECTION OF C9ORF72 MUTATION REVEALS MOTOR NEURON VULNERABILITY TO AMPAR-MEDIATED EXCITOTOXICITY

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Keywords: C9ORF72, CRISPR/Cas9, excitotoxicity

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by death of both upper and lower motor neurons (MNs). Intronic hexanucleotide GGGGCC (G4C2) repeat expansion is C9ORF72 gene is the most common cause of familial ALS (1). A powerful approach to study the pathomechanism of G4C2 repeats expansion in human MNs is use of human-induced pluripotent stem cells (iPSC) derived MNs from ALS patients carrying C9ORF72 mutation. Previous studies have implicated MN vulnerability to AMPA-receptor (AMPAR)-mediated excitotoxicity (2).

Objective: Gene correction of C9ORF72 mutation in hiPSC and assess if C9orf72 mutation causes MN vulnerability to AMPAR-mediated excitotoxicity.

Methods: MN’s were generated from iPSC derived from two healthy donors, two ALS patients harboring C9ORF72 mutation, and one gene corrected iPSC using standard protocol (3). All the lines differentiated into highly enriched spinal neuronal culture that expressed MN marker Isl1/2 (40–60%).

Results: A major challenge in disease modeling using hiPSC is the genetic variation including gene modifiers between individual donors. To overcome this challenge, we used CRISPR/Cas9 technology to excise G4C2 (750 repeats) from a C9ORF72 patient iPSC line thereby enabling us to directly study the role of pathogenic G4C2 repeats in iPSC derived MNs (C9-D). MN’s derived from both C9ORF72 iPSCs expressed pathogenic intranuclear RNA foci but was absent in both control and C9-D line. Exposure of AMPA (100 μM) resulted in a greater cell death in Week 3 C9orf72 mutant MN when compared to control and gene-corrected (C9-D) MNs. MN vulnerability to excitotoxicity was substantially reduced by the treatment with jorotoxin, a selective inhibitor of Ca2+-permeable AMPARs. Furthermore, at Week 3 C9ORF72 MNs demonstrated increased block of AMPAR-mediated currents by NASPM, a selective inhibitor of Ca2+-AMPAR, and elevated single-channel conductance when compared to control and C9-D MNs.

Discussion and conclusion: Generation of isogenic control iPSC from C9ORF72 mutant line demonstrates the causal link between the repeat expansion mutation and MN vulnerability to AMPAR-mediated excitotoxicity. Our data also shows that excitotoxicity is mediated by elevated expression of Ca2+-AMPAR in C9ORF72 MNs.

Acknowledgements: The Wellcome Trust, Euan MacDonald Centre

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DOI: 10.1080/21678421.2016.1232060/004

C9ORF72 REPEAT EXPANSIONS CAUSE AXONAL TRANSPORT DEFECTS IN IPSC-DERIVED MOTOR NEURONS

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Keywords: C9ORF72, iPSCs, axonal transport

Background: An intronic expansion of a GGGGCC repeat within the C9ORF72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Several downstream mechanisms have been proposed but the exact mechanism underlying the selective motor neuron degeneration is still unknown. Defective axonal transport is an early perturbed event in several neurodegenerative disorders, occurring prior to cell loss.
**Objectives:** Here, we have used induced pluripotent stem cells (iPSCs) derived from C9ORF72 FTLD/ALS patients and control fibroblasts in order to investigate the consequences of a C9ORF72 expansion in human motor neurons.

**Methods:** C9ORF72 and control iPSCs have been successfully generated using CytoTune-2 Sendai-virus reprogramming kit and further differentiated into motor neurons using a specific motor neuron differentiation protocol. Immunocytochemistry, gene expression and electrophysiological analysis have been performed to validate that the differentiation protocol yielded mature motor neurons. In order to measure the transport of mitochondria within the axons, MN cultures differentiated from control and C9ORF72 iPSCs were labeled with MitoTracker. Live cell imaging was used to monitor mitochondrial translocation along MN processes and time-distance kymographs were generated to quantify the number of stationary and moving mitochondria.

**Results:** No difference in the ability to differentiate into mature, functional, and Hb9/Isl1/ChAT positive motor neurons was observed between control and C9ORF72-positive cultures. C9ORF72 iPSCs-derived MNs showed an increase in p62/SQSTM1 levels compared to control. Moreover, we found that the C9ORF72 expansion resulted in a significant decrease in the number of motile mitochondria along the processes and this phenotype seemed to be more pronounced over time. An equal number of mitochondria in patient and control axons have been observed.

**Discussion and conclusion:** Our findings demonstrate that iPSC-derived motor neurons can reveal potential disease-relevant phenotypes and show that C9ORF72 repeat expansions cause axonal transport impairments in human-derived motor neurons.

DOI: 10.1080/21678421.2016.1232060/005

**P135 CHARACTERIZATION OF THE TDP-43 SPlicing TARGET TNIK IN NEURONAL DIFFERENTIATION**

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**Keywords:** TDP-43, TNIK, neuronal differentiation

**Background:** Cytoplasmic TDP-43 inclusions represent the neuropathological hallmark of ALS and of a subset of FTLD. Defects in RNA metabolism are mainly described in association to TDP-43 in the hypothesis of a loss-of-function mechanism. We have recently demonstrated that TDP-43 regulates the alternative splicing of several pre-mRNA targets, including TNIK, a Ser/Thr kinase with an important role in synaptic function, neurogenesis, and cytoskeleton dynamics. In condition of TDP-43 knock-down, the inclusion of the alternatively spliced exon 15 of TNIK gene increases as well as the corresponding protein isoforms (TNIKex15). Exon 15 encodes for a 29 amino acid sequence in the intermediate domain of the protein with unknown function.

**Objective:** We investigated the possible biological consequences of TDP-43 dysfunction by studying the regulation of the alternative splicing of exon 15 of TNIK gene, a TDP-43 splicing target, in different experimental human models.

**Methods:** In different human tissues, in human neuroblastoma SK-N-BE cells treated with retinoic acid and in human iPSCs differentiated into motoneurons, the alternative splicing of TNIKex15 was analyzed by both RT-PCR and WB using a custom antibody recognizing exon 15-encoded region. Regulation of TNIKex15 splicing was also assessed by a minigene splicing assay in HEK cells.

**Results:** We observed that TNIK alternative splicing is differently regulated in human tissues. TNIKex15 mRNA isoforms were prevalent in all cerebral (frontal cortex, hippocampus, cerebellum) and spinal cord regions analyzed as well as in skeletal muscle, as compared to lung and kidney where these isoforms were absent. When we induced neuronal differentiation in vitro, a significant increase of TNIKex15 isoforms, both at transcript and protein level, was observed in SK-N-BE treated with retinoic acid and in iPSCs differentiated into motoneurons. Immunofluorescence analyses showed a prevalent perinuclear distribution of TNIKex15 protein in neuron/motoneuron-differentiated cells. Since TDP-43 protein levels remained unchanged during in vitro neuronal differentiation, we focused on the possible involvement of other splicing factors in regulating TNIKex15 splicing. As putative consensus binding sequences for ELAV and NOVA1 proteins were present in intron 15 and as these RNA-binding proteins are specifically expressed during neuronal differentiation, we studied their effect on TNIKex15 splicing using a minigene assay. Upon over-expression of these single-splicing factors and in competition assays with TDP-43, we found that TNIKex15 inclusion is differently regulated by ELAV and NOVA1, suggesting a complex interplay between TDP-43 and these neuronal-specific splicing factors.

**Discussion and conclusion:** Our data show that alternative splicing of TNIK gene is differently regulated in human tissues and suggest a potential role of TNIKex15 isoforms in human brain and during neuronal/motoneuronal differentiation. Given the different subcellular localization of TNIKex15 protein, the specific function of these isoforms needs to be further investigated also in association to ALS/FTLD diseases.

DOI: 10.1080/21678421.2016.1232060/006
P136 ALS-CAUSING MISSENSE MUTATIONS OF CHCHD10 AFFECT PROTEIN STRUCTURE AND STABILITY

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Keywords: CHCHD10, mutations, interactions

Background: Coiled-coil helix coiled-coil helix domain containing protein 10 (CHCHD10) is a mitochondrial protein encoded in the nucleus. It is enriched at the cristae junctions of mitochondria and possibly plays a role in cristae structure, respiratory chain regulation (1) and mtDNA stability (2). The finding that mutations in CHCHD10 are associated with familial ALS and FTD directly links mitochondrial dysfunction to the pathology of the ALS-FTD continuum. However, the molecular pathogenesis of CHCHD10-related neurodegeneration remained so far enigmatic.

Objectives: In this study, we analyze the impact of three missense variants found in ALS patients (R15L, P34S, and G66V) on the structure and stability of CHCHD10.

Methods: CHCHD10 turnover was analyzed by the inhibition of translation of HEK293 cells overexpressing wild-type or mutant CHCHD10 followed by the determination of CHCHD10 levels by Western blotting. For structural analyses, we purified wild-type and mutant CHCHD10 expressed in E.coli and compared the respective tertiary structures by CD spectroscopy. Additionally, we took advantage of the recombinant CHCHD10 proteins and determined the thermal stability using a Thermofluor assay.

Results: Analysis of CHCHD10 protein turnover and thermal denaturation reveal a decrease in protein stability and an increased degradation of the R15L and G66V mutants, but not the P34S substitution compared to wild-type CHCHD10. These results are in line with CD spectroscopic measurements indicating considerable structural changes of the R15L and G66V mutant proteins. These structural changes are more pronounced under membrane-mimicking conditions.

Discussion and conclusion: These results match the most recent genetic evidence indicating that the R15L and G66V variants of CHCHD10 are pathogenic while P34S is not significantly associated with neurodegenerative diseases (3). We thus hypothesize that mutations of CHCHD10 induce a structural disturbance and loss-of-function of CHCHD10. Consequently, in order to elucidate downstream pathways mediating detrimental effects of CHCHD10 mutations, we performed a mass spectrometry-based screen for differential, disease-relevant binding partners of wild-type or R15L and G66V mutant CHCHD10, respectively.

Acknowledgements: This work was supported in whole or in part by grants from the German Federal Ministry of Education and Research (German network for ALS research (MND-NET; 01GM1103A)), the Charcot Foundation for ALS Research (Albert C. Ludolph, Jochen H. Weishaupt), the DFG-funded Swabian ALS registry and the International Graduate School in Molecular Medicine Ulm funded by the Excellence Initiative of the German Federal and State Governments.

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DOI: 10.1080/21678421.2016.1232060/007

P137 ALS-ASSOCIATED MUTATIONS IN MATRIN 3 ALTER PROTEIN–PROTEIN INTERACTIONS

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Keywords: Matrin 3, mass spectrometry, RNA binding proteins

Background: We recently described four mutations in the Matrin 3 gene encoding the nuclear matrix protein Matrin 3 that are associated with ALS (1). In human spinal cord tissue, Matrin 3 immunostaining is present in both motor neurons and glia, predominantly nuclear, and is stronger in ALS patients than in control cases and strongest in an ALS patient with a mutation in Matrin 3. Matrin 3 does not appear to exhibit gross mislocalization in ALS tissue as is often seen in other proteins involved in ALS; therefore we have chosen to explore its role and protein interactions within the nucleus.

Objectives: We sought to determine the protein binding partners of Matrin 3 as well as determine which of these interactions are altered by ALS causing mutations.

Methods: Cell lines stably expressing Flag tagged constructs of each of the four mutations and wild-type Matrin 3 were created in NSC-34 motor neuron-like cells. Immunoprecipitation experiments were performed in these cell lines followed by the separation by gel electrophoresis and tryptic digestion of excised bands. Peptides were extracted and analyzed on a Thermo LTQ Orbitrap Velos mass spectrometer. Interactions were confirmed with immunoprecipitation followed by western blot and colocalization analysis. Proteins binding to wild-type Matrin 3 were compared to proteins binding Matrin 3 mutant proteins to yield a list of protein interactions altered by ALS-linked mutations.
Results: In total, we identified 173 proteins meeting our confidence threshold that bound to one or more of the Matrin 3 constructs with hnRNPD having the highest confidence score for all five. After gene ontology analysis the top category found for both wild-type and all four mutations was mRNA metabolic processes. Other top categories included RNA splicing, RNA processing, and mRNA splicing though the order of such categories and the number of proteins found in each differed between wild-type and mutant. Our experiments confirmed Matrin 3 interacting proteins previously published as well as novel interactions with proteins altered in ALS pathogenesis such as FUS, Map1b, and hnRNPA3.

Discussion and conclusions: Mutations in Matrin 3 are novel to the field of ALS and neurodegeneration as a whole. This work gives us a better understanding of the many functions that Matrin 3 plays within the nucleus and cellular processes that rely on Matrin 3. This work is the first attempt at determining the numerous cellular processes disrupted by Matrin 3 mutations in a disease state. Moreover, we have discovered functional links between Matrin 3 and gene products that have mutations known to cause ALS and other neurodegenerative diseases, suggesting Matrin 3 may contribute to pathogenic mechanisms induced by these other disease causing mutations.

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DOI: 10.1080/21678421.2016.1232060/008

P138 CYTOPLASMICALLY MISLOCALIZED FUS EXPRESSED FROM THE ENDOGENOUS CRISPR/CAS9-MODIFIED GENE TRIGGERS SPONTANEOUS ASSEMBLY OF FUS GRANULES IN CULTURED HUMAN CELLS

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Keywords: RNA-binding proteins, RNA granules, protein aggregation

Background: A subset of familial ALS cases is characterized by FUSopathy – mislocalization and aggregation of mutant FUS protein in spinal neurons with its concomitant nuclear clearance. Most of the studies on FUS pathology carried out so far used cellular models with transient or constitutive overexpression of human FUS and its disease-linked variants. Although these have been instrumental to establish cellular processes affected by FUS malfunction, abnormally high FUS levels may cause various artefacts. Previously, we have shown that overexpression of certain ALS-linked FUS variants triggers formation of small granules in the cytoplasm, which shared some common properties but were distinct from stress granules (1).

Objectives: To assess whether normal cellular levels of cytoplasmically mislocalized FUS are sufficient to promote formation of such granules in cultured human cells.

Methods: CRISPR/Cas9 technology for genome editing was used to produce several single cell derived clones of human neuroblastoma SH-SY5Y' cells with deletion of gene sequences causing expression of FUS lacking nuclear localization signal (NLS), or with longer C-terminal truncation, corresponding to a known familial mutation, G466VfsX14. We also obtained a clone with completely abolished FUS production.

Results: In cells expressing FUS with deleted NLS, the protein was redistributed to the cytoplasm where it was readily recruited to stress granules upon stress exposure. In one of the lines, FUS-positive cytoplasmic granules were formed which possessed characteristics similar to granules previously observed in cells overexpressing ALS-linked FUS variants. Cytoplasmic accumulation and formation of FUS granules by FUS mutants could be promoted by proteasomal inhibition. Moreover, FUS granules could cluster together in response to stress and such higher-order assemblies persisted longer than normal stress granules after removal of stress.

Discussion and conclusions: These data indicate that the deficiency in FUS nuclear import alone, without an increase in the cellular protein levels, is sufficient to trigger and maintain spontaneous RNA-dependent aggregation of FUS protein in the cytoplasm.

Acknowledgments: The work was supported by Motor Neurone Disease Association (Buchman/Apr13/6096) and Russian Scientific Fund (14-14-01138). TS is a Medical Research Foundation/Medical Research Council (MRF/MRC) fellow. HA is supported by China Scholarship Council/Cardiff University PhD studentship.

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DOI: 10.1080/21678421.2016.1232060/009

P139 SERUM MICRORNA-PROFILES IMPLICATE NEW RNA-BINDING PROTEINS IN ALS

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Keywords: microRNA, RNA-binding proteins, serum

Background: In previous studies, we identified a subset of downregulated serum microRNAs (miRNAs) in familial ALS patients that was independent from the underlying disease gene and already evident in pre-clinical mutation carriers up to 20 years before the estimated onset of the
disease (1). Furthermore, similar miRNA-profiles were found in a majority (~60%) of sporadic ALS patients (2). Most strikingly, a common 5-nucleotide-sequence motif (GDCGG; D = G, A or U) was highly significantly enriched in the downregulated miRNAs. We therefore assume that the ALS-related downregulation of miRNAs is due to the deregulation/malfunction of one or several specific RNA-binding protein(s).

**Objectives:** To identify RNA-binding proteins associated with the GDCGG-motif of ALS-related miRNAs and to determine their role in ALS-pathogenesis.

**Methods:** We used miRNA-pulldown experiments in lysates of HEK293 cells followed by mass spectrometric identification and quantification of binding proteins. MiRNAs were designed to allow discrimination between unspecific RNA-binding proteins and proteins specifically binding the GDCGG-motif. Candidate proteins were purified from *E. coli* and HEK293 cells and analyzed for their RNA-binding properties using different biochemical assays. MiRNA-pulldown experiments in human serum were used to confirm the results in a physiological context.

**Results:** Mass spectrometry revealed 37 proteins closely associated with the GDCGG-motif. Biochemical analyses of the top three candidate proteins show direct binding of two of them to miRNAs containing the GDCGG-sequence. Physiological validation in human serum confirms binding of these two proteins to the ALS-related miRNAs in *vivo*.

**Discussion and conclusion:** We could identify two RNA-binding proteins interacting directly and specifically with the ALS-related miRNAs containing the GDCGG-motif. Downregulation of these miRNAs in ALS might indicate a deregulation/malfunction of these two proteins in ALS. As the miRNA-profiles were already evident in pre-clinical ALS mutation carriers, these findings might be related to a very early event in the pathogenic cascade. Further studies are needed to determine the roles of these RNA-binding proteins in ALS pathogenesis.

**Acknowledgements:** This work was supported by grants from the German Federal Ministry of Education and Research (STRENGTH consortium and BMBF; 01GI0704, German network for ALS research (MND-NET; 01GM1103A)), the Charcot Foundation for ALS Research (ACL, JHW), the virtual Helmholtz Institute "RNA-Dysmetabolism in ALS and FTD", and the DFG-funded Swabian ALS registry.

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DOI: 10.1080/21678421.2016.1232060/0010

**PI140 RAB1 REVERSES BOTH AUTOPHAGY DYSFUNCTION AND ER-GOLGI TRAFFICKING DEFECTS IN ALS**

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**Keywords:** trafficking, autophagy, Rab1

**Background:** Several diverse proteins are linked genetically or pathologically to neurodegeneration in amyotrophic lateral sclerosis (ALS) including superoxide dismutase (SOD1), fused in sarcoma (FUS), and TAR-DNA binding protein-43 (TDP-43). Mutant forms of these proteins inhibit protein transport between the endoplasmic reticulum (ER) and Golgi apparatus in neuronal cells (1), and cells expressing ALS-associated mutant SOD1, TDP, and FUS display autophagy defects. Rab1 has a pivotal role in mediating intracellular membrane trafficking events, including ER-Golgi trafficking (2,3) and autophagosome formation (4). However, the function of Rab1 in ALS remains unclear.

**Objectives:** To investigate the effect of Rab1 overexpression on (i) ER-Golgi trafficking and (ii) autophagy, in neuronal cells expressing ALS-associated mutant proteins, (iii) the distribution of Rab1 in motor neurons of human spinal cord tissues from patients with sporadic ALS, (iv) and to identify novel Rab1-mimetic compound that could have therapeutic benefit in ALS.

**Methods:** Cortical neurons and motor neurons isolated from SOD1G93A mice and neuroblastoma Neuro2a cells expressing ALS-associated mutant proteins, were transfected with mCherry-tagged temperature sensitive mutant vesicular stomatitis virus glycoprotein (VSVGts045) to examine ER-Golgi trafficking. Autophagosome formation was examined using immunocytochemistry for LC3. Paraffin-fixed spinal cord sections from patients with sporadic ALS were immunostained with anti-Rab1, anti-TDP-43, and anti-ubiquitin antibodies.

**Results:** Rab1 overexpression rescues inhibition of ER-Golgi transport and ER stress triggered by mSOD1, mTDP-43 and mFUS, and apoptosis and inclusion formation triggered by mSOD1. However, the inactive mutant Rab1S25N did not rescue ER stress, and the constitutively active Rab1Q70L was more protective relative to WTRab1. Rab1 also restored the inhibitory effects of mFUS on autophagosome and autolyosome formation. Rab1 formed inclusions in motor neurons of spinal cords from sporadic ALS patients and approximately 40% of the Rab1 inclusion-positive motor neurons...
colocalized with TDP-43. We are currently investigating whether novel compounds that mimic Rab1 activity are protective in neuronal cells expressing mutant SOD1, TDP43, and FUS.

**Conclusion:** We demonstrate that Rab1 rescued inhibition of ER-Golgi transport in cells expressing mutant SOD1, TDP-43, or FUS. This implies that restoring Rab1-mediated ER-Golgi transport and autophagy regulation is a novel therapeutic target in ALS. Finding a therapeutic compound which mimics Rab1 activity could be effective as a novel and broadly acting therapeutic agent in multiple forms of ALS.

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DOI: 10.1080/21678421.2016.1232060/0011

**P141 THE ROLE OF SPECIFIC PDI FAMILY MEMBERS IN PROTECTION AGAINST ALS-LIKE CELLULAR PATHOLOGIES**

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**Keywords:** PDI, protein misfolding, ERp57

**Background:** Proteostasis inhibition, inducing endoplasmic reticulum (ER) stress, protein misfolding, and proteasomal dysfunction, are all typical cellular pathologies associated with ALS. They are implicated in the formation of misfolded protein inclusions, which in almost all ALS patients contain misfolded TDP-43. These pathologies can be imitated in neuronal cultures by the overexpression of mutant TDP-43 or SOD1, thus creating a model for the study of proteostasis disruption in cells. Using this model, it has been established that an ER chaperone, protein disulfide isomerase (PDI), is protective against ER stress, protein misfolding, and apoptosis.

**Objectives:** A series of other ER chaperones, also from the PDI family, were examined for protection against misfolded proteins linked to ALS. These included ERp57, ERp72, and PDIA2.

**Methods:** Recombinant, tagged SOD1, TDP-43, and PDI family members were overexpressed in mouse motor neuron-like NSC-34 cells. Markers of cellular pathologies including cell death, inclusion formation, ER stress were assessed and measured using fluorescence microscopy.

**Results:** Results suggested that clear specificities existed in the protective activity. ERp57 was protective against cell death, ER stress, inclusion formation and ubiquitin proteasome system dysfunction in mutant SOD1 overexpressed cells. ERp72 and PDIA2 were protective against cell death but not against ER stress and inclusion formation. Similar protection against cellular pathologies induced by mutant TDP-43 was seen with ERp57, while in lumbar spinal cords from sporadic ALS patients, possible ERp57 colocalization with TDP-43-positive inclusions warrants further investigation.

**Discussion and conclusion:** These results suggest that, like PDI, ERp57 may have a protective role against ALS-like pathological events induced by both mutant SOD1 and TDP-43. This suggests that the PDI family has a broader therapeutic role in ALS than previously considered, and specific protein activities are associated with individual PDI members. The design of novel therapeutics based on the structure of PDI therefore may benefit from considering characteristics of the wider PDI family.

DOI: 10.1080/21678421.2016.1232060/0012

**P142 THE NUCLEAR PORE COMPLEX IS COMPROMISED IN ALS**

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**Keywords:** nuclear pore complex, nucleocytoplasmic transport, neurodegeneration

**Background:** Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease and Frontotemporal dementia (FTD) is the second most common form of early-onset dementia. Interestingly, both of these devastating neurodegenerative diseases share a common genetic mutation in chromosome 9 open reading frame 72 (C9orf72). An expanded hexanucleotide repeat (GGGGCC) in intron 1 of the C9orf72 gene is the most common genetic cause of familial and sporadic ALS and FTD. Recently, this gene has been sequenced in three independent labs published three papers including one from our own group simultaneously showing that dysfunction in Nucleocytoplasmic Transport (NCT) may be a fundamental pathway for C9orf72 ALS-FTD pathogenesis. NCT, the trafficking of protein and RNA between the nucleus and cytoplasm, is critical for signal transduction and is especially arduous for neurons due to their highly polarized biology. Efficient regulation of this process is mediated by the Nuclear Pore Complex (NPC), an extraordinary molecular machine that serves as the main gateway to the nucleus. In order for
any cell to function properly, it is imperative that RNA and protein be efficiently and selectively exchanged between the nucleus and the cytoplasm. This critical task is achieved by the ~2000 NPCs that span the entire nuclear envelope. Each NPC consists of multiple copies of 30 different proteins called Nucleoporins (NUPs) that differ in anatomical location, function, domain, post-translational modification and residence time. Mutations in various NUPs result in tissue-specific diseases. Additionally, some of the longest-lived proteins in the mammalian brain are specific NUPs and may represent the “weakest link” in the aging proteome.

Results: We now present data using human brain and iPS neurons that the NPC may also be compromised in sporadic ALS (sALS). We have surveyed the majority of NUPs in transgenic and BAC C9orf72 mice, iPS neurons/astrocytes, HEK293 cells and human post mortem brain tissue using IF, IHC, super resolution imaging, western blot, FRAP, shRNA, overexpression constructs, and proteomic analysis. We have identified a unique set of NUPs with critical and disease relevant functions that are consistently affected across not only models of C9orf72 but also sALS indicating that NPC dysfunction may be a common insult and pathogenic mechanism in the majority of ALS.

Discussion: This suggests that NPC dysfunction may be a critical global mechanism of neurodegeneration and what distinguishes one disease from another is the unique set of NUPs that are differentially affected.

Acknowledgments: National Science Foundation Graduate Research Fellowship, Johns Hopkins Solomon H. Snyder Department of Neuroscience Graduate Research Fellowship, Target ALS, NIH, ALSA, MDA, Robert Packard Center

DOI: 10.1080/21678421.2016.1232060/0013

P143 GLUCOSYLCERAMIDE AND GLYCOSPHINGOLIPIDS ARE PART OF THE RESPONSE TO MOTOR UNIT STRESS IN ALS

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Background: Autoimmune, an essential homeostatic mechanism, eliminates damaging entities from the cytoplasm. Interestingly, autoantigen does not randomly degrade cytoplasmic content; autoantigen receptors such as p62, NDP52, and NBR1 link specific cargoes to the nascent autophagosome membrane for degradation. Evidence suggests that mutations in selective autophagy pathways specialized in elimination of specific substrates cause distinct neurodegenerative diseases including...
Amyotrophic Lateral Sclerosis (ALS). Pathology is presumably mediated by accumulation of toxic autophagic substrates, but which autophagy substrates lead to ALS pathology remains uncertain.

**Objectives:** Historically, the field of autophagy has focused on proteins and organelles as the substrates for degradation, despite evidence that a major fraction of RNA is degraded by autophagy, particularly in stress. Mutated versions of several RNA-binding proteins linked to ALS (e.g. FUS, TDP-43) cause formation of RNA-rich granules called stress granules (SGs). We hypothesized that SGs and another type of RNA granule - processing bodies (PBs) are selectively targeted for the degradation by autophagy receptors.

**Methods and results:** We found that p62 and NDP52 are preferentially recruited to SGs and PBs, respectively, as detected by expressing mCherry -TIA-1 and GFP-Dcp1a. Further NDP52 preferentially colocalized with endogenous markers of PBs rather than SGs, while p62 colocalized with endogenous SGs induced by oxidative stress. A third autophagy receptor – NBR1, colocalized with a subset of these SGs and PBs. Proteomic analyses of p62 interactors during oxidative stress revealed an extensive network of RNA-binding proteins localized to SGs. Association of p62 with RNA-binding proteins localized to SGs was confirmed by immunoprecipitation in ALS patients as well. Further, differential biochemical profiling of ALS cerebellum tissues enriched for SG proteins along with p62 in the insoluble urea fraction. PBs and SGs frequently colocalized with autophagosomes and depletion of NDP52 and p62 increased the number of PBs and SGs per cell, respectively. Overall, this strongly suggests that individual autophagy receptors preferentially recognize distinct RNA granules (PBs or SGs) for selective degradation by autophagy (1).

**Discussion:** Autophagy may be an effective mechanism to regulate bulk turnover of RNA regrouped in granules. Intriguingly, emerging evidence suggests that pathology in ALS involves inefficient autophagic clearance of mutant proteins in RNA-rich SGs. Our identification of p62 as a selective autophagy receptor for degradation of SGs suggests a mechanism for the genetic implication of p62 in ALS and underscores the physiological relevance of RNA degradation by autophagy.

**Acknowledgements:** (i) Canadian Institutes of Health Research; (ii) ALS Society of Canada: 2015 ALS Cycle of Hope Doctoral Research Award; (iii) Vanier Canada Graduate Scholarships; (iv) We thank Dr Janice Robertson for kindly providing us with cerebellum extracts of C9ORF72 and sALS patients.

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PI45 THE TGF-ß SYSTEM AS AN IMPORTANT MEDIATOR IN THE DISEASE PROGRESSION OF ALS

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**Keywords:** amyotrophic lateral sclerosis, TGF-ß pathway, fibrosis

**Background:** Neurodegenerative disorders including amyotrophic lateral sclerosis (ALS) exhibit as a specific characteristic an enhanced pro-inflammatory milieu with increased liquor concentrations of Transforming Growth Factor beta (TGF-ß). In combination with promoting the degeneration of neurons, these immunological alterations inhibit neurogenesis and drive stem cell quiescence. Despite an increasing knowledge and continuously growing research interest, the heterogenic etiology of this disorder in combination with a lack of validated biomarkers aggravates the effective treatment of this “orphan disease.”

**Methods:** Here, we first investigated the activation state of the endogenous TGF-ß system in post mortem spinal cord (SC), motor cortex (MC), and occipital lobe (OL) homogenates (kindly provided by Prof. Dr Thal and Prof. Dr Petri, both MND network Germany) from ALS patients and controls. Therefore, the expression patterns of TGF-ßRI,II,III, the ligands TGF-ß1, TGF-ß2 and the most important downstream molecules p-Smad 2/3, Smad 4, Smad 1/5, Smad 8 were determined via qRT-PCR and Western Blot analysis. CTGF as an important downstream-molecule within the TGF-ß system mediates the fibrotic effects of TGF-ß by inducing the deposition of ECM and modulating the reorganization of actin-cytoskeleton. Since these pathogenic modifications of the extracellular matrix (ECM) and the actin-cytoskeleton often correlate with disease progression and disease severity, the expression levels of connective tissue growth factor (CTGF) were analyzed within the tissue mentioned earlier. In addition, to investigate whether fibrosis is involved in the progression of ALS, we examined the expression profile of the two main components of ECM, Fibronectin (FN), and Collagen IV (CollIV). The differences of the ECM and the actin-cytoskeleton of ALS patients and controls within the three different tissues were obtained by qRT-PCR, Western Blot analysis, and immunofluorescence.

**Results:** We were able to demonstrate enhanced mRNA as well as protein levels of TGF-ß2 exclusively in postmortem SC tissue of ALS patients compared to healthy controls. Within the MC and OL tissue, there was no alteration in the expression levels of TGF-ß1, TGF-ß2, and the TGF-ßRII were detectable.

**Discussion and conclusion:** Taken together, the results of the current study might shed some light on possible pathways mediating disease progression and provide possible purchases for the treatment options in ALS.

**Acknowledgements:** Spinal cord and motor cortex tissue was kindly provided by MND Network Germany.

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**Keywords:** amyotrophic lateral sclerosis, TGF-ß pathway, fibrosis
P146 IMPAIRED ACTIVATION OF ALS MONOCYTES BY EXOSOMES

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Keywords: monocyte, exosome, TDP-43

Background: Neurodegeneration in amyotrophic lateral sclerosis (ALS) is accompanied by a well characterized neuroinflammatory reaction within the central nervous system (CNS) and importantly, also cells of the peripheral immune system (1). Particularly, circulating monocytes have been implicated in ALS pathogenesis (2). Exosomes are membrane-enclosed vesicles secreted by various cell types with a diameter of 50–150 nm (3).

Objectives: We hypothesize that circulating blood exosomes are putative mediators of the monocyctic deregulation observed in ALS patients. The aims of this study are to shed light on the interplay of peripheral blood monocytes and exosomal vesicles in the context of ALS.

Methods: We characterized the immunogenic content of serum exosomes isolated from healthy donors as well as ALS patients. Further, we applied exosomes isolated from human serum and TDP-43 containing exosomes produced in cell culture to cultures of primary human monocytes isolated from the blood of ALS patients and healthy age-matched controls. Subsequently we analyzed the monocyctic uptake of the applied exosomes as well as their consequent immunologic activation.

Results: Here, we found the uptake of serum exosomes by CD14++ monocytes to be independent of the exosome donor. Further, we show that pro-inflammatory cytokine secretion by ALS monocytes upon exosomal stimulation is impaired compared to control monocytes. Moreover, we show that both the exosomal uptake and the consecutive monocyctic cytokine secretion are dependent on the exosomal TDP-43 cargo.

Discussion and conclusions: We show that ALS monocytes exhibit an impaired pro-inflammatory reaction upon contact to serum exosomes at similar exosomal uptake efficiencies implicating a distinct functional impairment of ALS monocytes (1,2). Further, we provide evidence that the exosomal content of TDP-43 significantly affects the consecutive monocyctic activation. Thus, this study underlines the distinct functional deregulation of peripheral monocytes in ALS (2) and the impact of circulating blood exosomes on monocyte activation.

Acknowledgements: This research was supported by the Thierry Latran Foundation.

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DOI: 10.1080/21678421.2016.1232060/0017

P147 BLOOD-CSF BARRIER DISRUPTIONS IN ALS

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Keywords: choroid plexus, blood-brain barrier, cellular adhesion

Background: The blood-CSF (BCSF) barrier is structurally comprised of an endothelial cell layer and a polarized epithelial cell layer named the choroid plexus (CP). The BCSF barrier lines the inside of the brain ventricles, separating the blood in the vascular system from the cerebrospinal fluid (CSF) that is in direct contact with neurons. The CP mainly functions in CSF production and turnover as well as selective transport of nutrients from the systemic compartment, removal of metabolic products out of the brain, regulation of cell trafficking into the CSF, and the prevention of diffusion of harmful blood solutes into the brain. Under inflammatory conditions, the CP epithelium expresses many factors involved in mediating and facilitating an immune response thus serving as a primary point of entry of immune cells into the nervous system. Aging and neurodegeneration, specifically Alzheimer’s disease, have been reported to greatly affect CP morphology and function, decreasing CSF production and turnover by as much as 50%, altering levels of proteins involved in energy production and free radical scavenging, and increasing protein leakage from blood to the CSF. ALS is similarly associated with increased oxidative stress markers, neuronal loss, and metabolic disturbances that may also impede CP function. Our group and others have reported increased levels of many proteins in CSF from ALS patients, including inflammatory targets, cytoskeletal and extracellular matrix proteins, as well as aggregated proteins. Having these proteins and factors abnormally localized to the CSF compartment suggests impaired BCSF barrier permeability, increased protein leakage from blood to the CSF and decreased clearance of metabolic products. These in turn point to a dysfunctional BCSF barrier. To date, there have been few studies investigating BCSF barrier or CP alterations in ALS patients. We hereby have investigated BCSF integrity in ALS.

Results: We hypothesized that this physical barrier is disrupted in ALS possibly via excess metalloproteinase (MMP) activation, thus allowing an influx of immune cells into the CSF and conversely into the nervous system. We have investigated the morphology and distribution of various cell junction and cell adhesion proteins such as cadherins, occludins, and claudins in ALS CP compared to control CP by immunohistochemistry. We show that levels and distribution of many of these markers is altered in ALS. Results were confirmed by real-time PCR...
analysis. In addition, we have examined levels of various matrix metalloproteinases (MMP) in ALS CP compared to controls, as a potential cause for BCSF barrier breakdown.

Discussion: By investigating BCSF barrier disruption in ALS patients, our study provides novel mechanisms of how toxic metabolites and immune modulators spread to the CSF potentially providing new targets for therapy development.

DOI: 10.1080/21678421.2016.1232060/0018

P148 SYNAPSE LOSS: AN UNDERLYING CORRELATE OF COGNITIVE DECLINE IN AMYOTROPHIC LATERAL SCLEROSIS?

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Keywords: cognition, synapse, degeneration

Background: Approximately 50% of amyotrophic lateral sclerosis (ALS) patients exhibit cognitive and behavioral deficits. Most common, are deficits in verbal fluency and executive function, which are mediated by the frontal lobe. These deficits are similar to those observed in a seemingly disparate disorder, frontotemporal dementia (FTD), a disease caused by breakdown of the frontal and temporal lobes. Genetic studies suggest that ALS and FTD may lie on a disease spectrum. Repeat expansions in the C9orf72 gene result in either ALS or FTD and TDP-43 +ve protein aggregates are found in both diseases. Furthermore, significant overlap in cognitive dysfunction is observed in both patient cohorts and some FTD patients exhibit motor deficits, reinforcing the hypothesis of a disease spectrum.

Objective: Synapse loss is a common pathogenic feature of many neurodegenerative disorders, including FTD and ALS. Using high-resolution imaging techniques, we aimed to discover if this was also true for ALS and whether synapse loss in the frontal cortex may play an important role in the pathogenesis of cognitive decline in ALS patients.

Methods: We used the high-resolution imaging technique array tomography, to analyze approximately half a million synapses in the frontal cortex, from 11 control cases and 17 ALS cases. Of the 17 ALS cases analyzed, 11 were cognitively tested, 7 were unimpaired, and 4 cognitively impaired. Cognitive testing was performed using the Edinburgh Cognitive and Behavioral ALS Screen (ECAS), to give us an accurate representation of ALS-specific cognitive changes.

Results: There was a significant decrease (p<0.05) in mean synapse density in ALS frontal cortices (n=17) compared to control (n=11). When the ALS group was split into impaired (n=4) and unimpaired (n=7) groups, the impaired group had a lower synapse density in the frontal cortex than control nondemented brains (p<0.05).

No change in cortical thickness was noted between groups (p=0.4). In the motor cortex, no change in synapse density was observed between groups, however, the ALS motor cortices were significantly thinner (p=0.004). This suggests cortical atrophy of the motor cortex occurs in ALS cases and the tighter packing of synapses in the smaller cortex likely leads to no change in synapse density when compared to control. In a separate group of control (n=6) and ALS cases (n=20, not cognitively defined) electron microscopy revealed a decrease in synapses from the frontal cortex of ALS brains compared to controls (p=0.003). Furthermore, an increase in degenerating (electron dense) synapses was discovered in the ALS brains (p=0.025).

Discussion: Taken together, our data suggests that synapse loss in the frontal cortex may play an important role in the pathogenesis of cognitive decline in ALS patients.

Acknowledgements: Wellcome Trust (Institutional Strategic Support Fund), Medical Research Council, Medical Research Scotland, MND Scotland, Alzheimer’s Research UK/Chief Scientists Office Scotland, Euan Macdonald Centre and Sylvia Aitken Charitable Trust.

DOI: 10.1080/21678421.2016.1232060/0019

P149 NEURONAL SENESCENCE AS A CONTRIBUTOR TO NEURODEGENERATION

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Keywords: senescence, DNA damage response, secretory phenotype

Background: Senescent cells-associated secretory phenotype (SASP) may contribute to neurodegeneration. Recent findings in vitro suggest neurons undergo a senescent-like state associated with persistent DNA damage (1). Moreover, increased expression of a neuronal DNA damage response (DDR) associates with cognitive decline in an aging cohort (2), suggesting the neuronal SASP in response to persistent DNA damage may contribute to neurodegeneration.

Objectives: To determine whether neuronal senescence is a feature of motor neuron disease (MND) and to establish an in vitro neuronal model of persistent DNA damage.

Methods: Using immunohistochemistry, markers of DNA damage (H2AX) and senescence (p16, p21, and SA-β-gal) were investigated in frontal cortex, motor cortex, and spinal cord sections of 10 human autopsy MND and 10 control cases. A model of acute and chronic DNA damage was developed in vitro by stressing postmitotic LUHMES cells with hydrogen peroxide. Changes in the expression of DNA damage and senescence-related genes were investigated by qRT-PCR. GFP-LUHMES were cocultured with stressed LUHMES or...
incubated with their conditioned media (SCM) for 24 h; neurite outgrowth impairment of GFP-LUHMES was investigated.

**Results:** SA-β-gal activity was present in glia and neurons of both MND and control cases. Expression of p16 was detected in glia, whereas p21 was detected in glia and neurons. Glial p16 expression and neuronal p21 expression were significantly elevated in MND versus control cases (p=0.017 and p=0.029) in the frontal cortex. In the in vitro model, the detection of H2AX foci in stressed neurons confirmed oxidative damage in the form of DNA double-strand breaks (DSBs). Damage was repaired 24 h post-stress in the acute model, whereas in the persistent model, H2AX foci were detectable 96 h post-stress. Changes in the relative expression of senescence related genes were detected in both models, including upregulation of CDKN1A (p21). A reduction in the neurite length of GFP-LUHMES was detected when incubated with SCM (p<0.05); however, a more intense effect in neurite outgrowth impairment was seen under coculture conditions (p<0.0001).

**Conclusions:** A persistent DNA damage response is a feature of neurons and glia in MND. The differential expression of senescence-associated markers suggests that pathways differ between cell types. Acute and persistent DNA damage can be induced in postmitotic neurons in culture. Oxidative DNA damage induces senescence-related genes and these changes depend on the chronicity of the damage. Persistent DNA damage may impact cell functionality, including the release of damaging factors.

**Acknowledgements:** This project was supported by the British Neuropathological Society and Consejo Nacional de Ciencia y Tecnología, Mexico. Tissue was obtained from the Sheffield Brain Tissue Bank.

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DOI: 10.1080/21678421.2016.1232060/0020

**P150 A PROTEOMIC PERSPECTIVE: AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA DISEASE OVERLAP**

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**Keywords:** Proteomics, Frontotemporal dementia, C9orf72

**Background:** Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are progressive neurodegenerative diseases with substantial clinical, pathological, and genetic overlap. A hexanucleotide repeat expansion in the C9orf72 gene is the most frequent reported genetic cause of ALS and FTD. The objective of this study was to identify pathways involved in disease pathogenesis that are unique to each clinical phenotype and those that are similar across this spectrum.

**Methods:** We performed an unbiased, quantitative proteomic screen using post-mortem brain tissue from patients clinically diagnosed with ALS (n=19), FTD (n=12), and patients who had both ALS and FTD (n=10), compared to normal (without neurological disease) controls (n=10). Patient tissue included those with and without the C9orf72 expansion mutation.

**Results:** Our data identified several pathways that differentiated these four clinically defined groups using weighted correlation network analysis. These included RNA-binding proteins, astrocytic markers, proteins involved in nucleocyttoplasmic transport, and synaptic proteins among other pathways. Using principal component analysis, we found that the proteomic signatures segregated out by clinical diagnosis. The presence of a C9orf72 expansion mutation was not identified as an independent variable associated with proteomic differences. Future detailed validation of these observed differences will clue us into the molecular underpinnings of these overlapping diseases.

**Discussion:** Based on our findings, we conclude that there are overlapping cellular pathways implicated in the diseases along this spectrum but also specific differences unique to clinical phenotypes separating ALS, FTD, and ALS-FTD.

DOI: 10.1080/21678421.2016.1232060/0021

**P151 DETERMINING THE COMPOSITION OF FTLD INCLUSIONS BY SPATIALLY TARGETED OPTICAL MICROPROTEOMICS (STOMP)**

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**Keywords:** Frontotemporal dementia, proteomics, protein inclusions

**Background:** Frontotemporal lobar degeneration (FTLD) exhibits high genetic, phenotypic, and neuro-pathological heterogeneity. The presence of intraneuronal inclusions is a common pathological feature of all FTLD; the inclusions stain for either tau or ubiquitin. Ubiquitin positive cases also stain for TDP-43, FUS, or the dipeptide product of C9ORF72 hexanucleotide repeat expansions. The three major phenotypic forms of FTLD present as changes in personality and behavior (Frontotemporal Dementia), difficulty with language (Primary Progressive Aphasia) or loss of semantic knowledge (Semantic Dementia). Three genetic causes of FTLD include mutations in MAPT (gene encoding tau), GRN (progranulin gene), and C9ORF72. Remarkably, there is no direct correlation between phenotype, genotype, and pathology.
Objectives: We use a novel method to examine the proteomic composition of intraneuronal inclusions in human FTLD cases to identify unifying mechanism of FTLD. Determining FTLD proteomes may also provide insight into the mechanisms of ALS/MND, where diseased tissues contain inclusions composed of proteins also found in FTLD.

Methods: Currently, there are no comprehensive proteomic studies of FTLD inclusions. Methods that isolate inclusions by centrifugation tend to exclude soluble proteins, while laser capture microdissection is prone to sample contamination by the surrounding tissue due to the small size of the inclusions. We have developed an unbiased approach to elucidate FTLD inclusion proteomics in formalin-fixed brains called spatially targeted optical micro-proteomics (STOMP). In STOMP, the tissue section is saturated with a "photo-tag" compound. The tissue is stained with an antibody (anti-tau or anti-ubiquitin) to reveal the inclusions and imaged by standard confocal microscopy. The resulting image is a 3D coordinate file indicating the location of inclusions in the tissue. That image file in combination with two-photon excitation is used to deliver UV light specifically to the protein inclusions, which results in the exclusive attachment of the "photo-tag" to all proteins present in the inclusion bodies. The tissue section is then solubilized and the photo-tagged proteins are affinity-purified and identified by mass spectrometry. STOMP provides high resolution microproteomics for protein inclusions in FTLD at a resolution that far exceeds current methods.

Results: We have acquired 12 FTLD-ubiquitin cases, one case of FTD-tau, and 12 control cases from the Maritime Brain Tissue bank. As a preliminary result, we have performed STOMP on the FTLD-tau case and generated a list of proteins that associate with tau-positive inclusions. The list of proteins is pending statistical analysis and validation by immunohistochemistry; but the preliminary data show that STOMP is an effective method to interrogate FTLD inclusion proteomics.

Conclusions: Understanding the composition of protein inclusions in FTLD will increase our understanding of this heterogeneous group of disorders and our STOMP technique is likely the only existing technique that is capable of achieving this goal.

DOI: 10.1080/21678421.2016.1232060/0022

P152 FUNCTIONAL ANALYSIS OF MIR-1234-3P AND MIR-1825

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Keywords: miRNA, mRNA

Background: MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression. Increasing evidence suggest a dysregulation of miRNAs in ALS pathogenesis. Recently, we and others identified a significant downregulation of miR-1234-3p and miR-1825 in the serum of sporadic ALS (sALS) patients (1,2). Additionally, downregulation of miR-1825 was evident in the serum of familial ALS (fALS) cases (3). However, miR-1825 and miR-1234-3p are not well characterized and their mRNA targets have not been investigated so far.

Objectives: We aimed to examine if the extracellular downregulation of miR-1825 and miR-1234-3p in ALS patients is the consequence of reduced intracellular expression levels and to determine the possible role of miR-1825 in the pathogenesis of ALS.

Methods: Using RT-qPCR we measured the relative levels of miR-1825 and miR-1234-3p in various post-mortem tissue (brainstem, spinal cord, liver, lymph node, skeletal muscle) and ALS patient-derived keratinocytes. Additionally, we identified the mRNA targets of miR-1825 by combining both proteomic (mass spectrometry) and transcriptomic profiling (microarrays) in HEK293 cells transfected with a miR-1825-mimic. Targets of miR-1825 relevant for ALS were validated using a luciferase-approach.

Results: A significant downregulation of miR-1825 was detected in postmortem tissue of ALS patients while miR-1234-3p showed less prominent intracellular alterations. Several of the targets of miR-1825 were related to ALS and could be confirmed in vitro.

Discussion and conclusion: Our results confirm previous studies showing a systemic downregulation of miR-1825 in ALS (1,3). Knowledge of the targets of miR-1825 will help to clarify its role in ALS pathogenesis.

Acknowledgments: This work was supported by grants from the German Federal Ministry of Education and Research (STRENGTH consortium and BMBF; 01GI0704, German network for ALS research [MND-NET; 01GM1103A]), the Charcot Foundation for ALS Research (ACL, JHW), the virtual Helmholtz Institute “RNA-Dysmetabolism in ALS and FTD,” and the DFG-funded Swabian ALS registry

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DOI: 10.1080/21678421.2016.1232060/0023
P153 ERBB4, A CAUSATIVE GENE PRODUCT OF FAMILIAL ALS (ALS19), ACCUMULATES IN THE NEURONS AND OLIGODENDROCYTES IN PATIENTS WITH FRONTOTEMPORAL DEMENTIA

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Keywords: ErbB4, frontotemporal dementia, tau

Background: ErbB4 was identified as a causative gene for a rare familial ALS designated as ALS19 (1). We previously reported that ErbB4 expression decreases in the spinal motor neurons of sporadic ALS patients. A recent study also showed decreased ErbB4 expression in the motor neurons of SOD1 transgenic mice (2). Furthermore, accumulating evidence suggests that ErbB4 signaling insufficiency is related to other neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease (3–5).

Objectives: This study investigated the relevance of ErbB4 to frontotemporal dementia (FTD).

Methods: We immunohistochemically analyzed the brains of FTD patients (including Pick’s disease (n=1), corticobasal degeneration (n=4), progressive supranuclear palsy (n=3), argyrophilic grain disease (n=2), and FTLD17 (n=2)) by using a polyclonal anti-ErbB4 antibody raised against the intracellular C-terminal region of ErbB4 to evaluate the expression of ErbB4 in affected brain regions. This is an ongoing research study.

Results: ErbB4 accumulates in the pathological hallmarks of FTLD-TAU. These include Pick bodies in Pick’s disease, ballooned neurons and coiled bodies in corticobasal degeneration, globbose neurofibrillary tangles and coiled bodies in progressive supranuclear palsy, grains and coiled bodies in argyrophilic grain disease and various hyperphosphorylated tau inclusions in FTLD17. In contrast, astrocytic tau inclusions were not stained with anti-ErbB4 antibody.

Discussion and conclusions: This study showed that ErbB4 localizes to neuron- and oligodendroglia-derived pathological hallmarks in FTLD-TAU. Based on these findings, we hypothesize that ErbB4 colocalizes with abnormally phosphorylated tau causing impaired ErbB4 signaling. An alternative hypothesis is that the increased expression of ErbB4 and upregulation of its signaling cascade might accelerate the phosphorylation of tau. In summary, ErbB4, a causative gene product of familial ALS, might be involved in the pathophysiology of FTLD-TAU.

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DOI: 10.1080/21678421.2016.1232060/0024

P154 INVESTIGATING THE HISTOPATHOLOGICAL SIGNATURE OF BULBAR-ONSET ALS

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Keywords: bulbar ALS, disease propagation, histopathology

Background: A number of studies propose that bulbar-onset ALS (B-ALS), compared with limb-onset ALS (L-ALS), may present with a unique neurodegenerative profile that is associated with specific and concomitant cognitive/language impairments (1). Other studies report no such association (2). Theories about propagation of neuropathological changes in ALS progression are also debated. Two predominant hypotheses postulate that: (1) disease propagates from initial lesion site to proximate neuroanatomical regions (3); or (2) propagation occurs within neuronal networks that form functional and structural connectomes (4). Recent literature supporting the connectome hypothesis predominates (5).

Objectives/Design: To investigate regional histopathological features of cases with B-ALS vs. L-ALS in order to investigate the connectome hypothesis in the context of bulbar disease. We hypothesized that brain structures associated with bulbar motor/speech dysfunction such as Broca area, Wernicke area, primary auditory cortex, bulbar motor cortex, and subcortical white matter may demonstrate greater histopathology disease burden in B-ALS than L-ALS.

Methods/Analysis: Brain tissue samples from 5 B-ALS, 5 L-ALS, and 5 sex- and age-matched controls, all right-handed, were examined. Both ALS groups were matched by disease duration and premorbidly well characterized clinically with respect to bulbar motor and extramotor involvement and genetic workup. Histopathological evaluation included a central pathology review of left
hemispheric regions of interest (ROIs): Broca and Wernicke areas, primary motor, premotor, supplementary motor cortices, and primary auditory cortex, as well as brainstem motor nuclei, cerebellum, and white matter tracts (specifically arcuate and uncinate fasciculi, corticospinal, and corticobulbar tracts). Currently, all ROIs for ALS cases have been sampled and pathologic analysis is underway using semi-quantitative assessment of routine and immunohistochemical stains to determine neuronal loss (NeuN), gliosis (GFAP), macrophage/microglial density (CD163), proteinopathy (TDP43), axonal density (NF), and myelin pallor (HE/LFB).

Discussion: This ongoing work will result in better understanding of bulbar ALS from its clinical presentation to its neuropathology. Findings will shed light on mechanisms of pathology propagation in the disease, aid in accurate patient subtyping, and help predict disease progression, all paramount for discovery of the etiology and treatment for this currently incurable disease.

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DOI: 10.1080/21678421.2016.1232060/0025

P155 HUMAN MUTATIONS HELP CONNECT GENES TO PATHWAYS AND NETWORKS, AND OFFER A MECHANISTIC INSIGHT FOR SELECTIVE MOTOR NEURON VULNERABILITY

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Keywords: human mutations, selective vulnerability, motor neuron biology

Background: Mutations in many genes have been characterized as either “causative” or associated with ALS, HSP/PLS, and SMA pathology in patient populations. Despite mutations occurring in different genes, only the selected populations of neurons degenerate while others are spared. For example, corticospinal motor neurons (CSMN) display primary vulnerability in HSP/PLS, spinal motor neuron (SMN) loss is prominent in SMA, and in ALS both CSMN and SMN undergo progressive degeneration. By investigating the binding partners of disease-causing and associated mutations identified in human patients, we linked individual gene products and protein–protein interactions, thus revealing critical pathways, cellular events and individual proteins disrupted in the context of motor neuron disease. This helped us define cellular events, canonical pathways, key upstream effectors, and protein network interactions that are common and unique among diseases, and are responsible for selective motor neuron vulnerability.

Objectives: To identify protein–protein interactomes for ALS, HSP/PLS, and SMA and to reveal canonical pathways, proteins, and cellular functions critical for selective vulnerability of CSMN and SMN, and to further delineate commonalities and differences among each motor neuron disease.

Methods: We first compiled a published list of mutations causative or associated with ALS, HSP, PLS, and SMA, then determined the binding partners of each mutated gene product via curated and published information using large-data management tool boxes, such as ingenuity pathway analysis. After protein interactome for each disease was determined, we investigated the common and unique proteins, interaction domains, important canonical pathways, signaling molecules, upstream regulators, and protein–protein network interactions that are associated with each disease.

Results: To date, causative and associated genes have been identified in ALS (n=23; n=28), HSP/PLS (n=36; n=29), and SMA (n=4; n=17). ALS (n=1139), HSP/PLS (n=611) and SMA (n=672) binding partners were identified and among these binding partners common and unique proteins, proteins with most frequent interactions with mutated gene products were identified. We further identified canonical pathways, networks and individual proteins within these networks exhibiting the greatest number of interactions for all ALS, all HSP/PLS, and all SMA binding partners as well as disease exclusive and overlapping partners for each motor neuron disease. Our ongoing studies focus on the secreted molecules that are common and unique among diseases.

Conclusion: We conclude that human mutations inform us on the basis of selective motor neuron vulnerability. We find that it is not the individual gene, but the protein–protein network associated with each gene product that is responsible for the emergence of selective vulnerability and progressive degeneration in the context of ALS, SMA, and HSP/PLS. The information we generated has begun to reveal the most important cellular events that are perturbed and become dysfunctional in the presence of mutations. Identification of common and unique pathways and protein interaction dynamics important for distinct motor neuron population will help build effective treatment strategies in the near future.

DOI: 10.1080/21678421.2016.1232060/0026
P156 SELECTIVE MOTOR NEURONE VULNERABILITY IN SMA

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Keywords: selective vulnerability, spinal muscular atrophy

Spinal muscular atrophy is a childhood familial motor neurone disease (MND) characterized by loss of motor neurones (MNs). However, a growing body of evidence indicates that MNs have different vulnerability to the disease (1,2). Furthermore, such selective vulnerability seen in SMA is irrelevant to any morphological characteristics (1). We put forward the hypothesis that intrinsic molecular differences between motor neurones could modulate the disease severity.

Objectives: Our aim is to identify the intrinsic disease modifiers between motor neurones with different vulnerability.

Methods: Based on previous results (1), the motor neurones innervating extensor digital longus (EDL, resistant), gastrocnemius (GS, intermediate), and anterior tibialis (TA, vulnerable) were selected for this study. The motor neurones were retrogradely labeled in normal FVB mice (2 weeks age) with WGA-HRP and isolated with laser capture dissection (LCM) for transcriptome microarray analysis. Data mining of probe expression values using DAVID and GSEA created a comprehensive interpretation of the differences between the motor neuron groups. The potential disease modifying factors were subjected to in vivo and in vitro functionality validation.

Results: In this study, we directly accessed the transcriptional profiles of different motor neurones innervating different muscles by using microarray combined with laser capture microdissection. It enabled us to uncover subtle but important differences that may differentiate the vulnerabilities among these three groups of motor neurones. Additionally, wild-type mice were used to eliminate the false-positive result that may be secondary to the general disease response. Overall, we find resistant motor neurones display higher activities of cellular metabolisms such as oxidative phosphorylation, protein synthesis, and degradation.

Discussion and conclusions: Motor neurones cope with the disease differently. As such, it is believed that the understanding of intrinsic differences between more and less vulnerable motor neurones is the key to identifying protective pathways that could be therapeutic targets.

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DOI: 10.1080/21678421.2016.1232060/0027

P157 A DYNC1H1 MUTATION IN AUTOSOMAL DOMINANT SPINAL MUSCULAR ATROPHY SHOWS THE POTENTIAL OF PHARMACOLOGICAL INHIBITION OF HISTONE DEACETYLASE 6 AS A TREATMENT FOR DISEASE ASSOCIATED CELLULAR PHENOTYPES

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Keywords: microtubule acetylation, golgi apparatus, cytoplasmic dynein

Background: Spinal muscular atrophy with lower extremity predominance (SMA-LED) is an autosomal dominant congenital motor neuron disease. The condition presents with distal limb weakness and muscle atrophy, further compounded with intellectual disability. The most common cause are mutations in dynein cytoplasmic 1 heavy chain 1 (DYNC1H1; OMIM:600112), which encodes the largest subunit of cytoplasmic dynein 1. Dynine is defined by its role as a retrogradely oriented molecular motor but it is also fundamental to other cellular processes including growth cone dynamics and regulation of the Golgi apparatus. Moreover, mutations in dynactin 1 (DCTN1; OMIM: 601143) encoding p150 (Glued) subunit of the dynactin complex, which regulates cytoplasmic dynein function, cause autosomal dominant distal hereditary motor neuronopathy.

Objective: To dissect common molecular mechanisms underlying motor neuron degeneration caused by R399G and D338N mutations in DYNC1H1.

Methods: Immunofluorescence was performed on patient fibroblasts harboring the R399G or D338N DYNC1H1 mutation to assess the integrity of the Golgi apparatus and the localization of dynein to the organelle. Modifications
of microtubules and the interaction of dynein with golgin-160 were investigated using biochemical analysis.

**Results:** Decreased α-tubulin acetylation was a common molecular phenotype in patient fibroblasts harboring the R399G (p<0.05, N=3) or D338N (p<0.01, N=5) mutation in comparison to wild-type fibroblasts (N=3 and N=5, respectively). However, only the R399G mutant fibroblasts (N=20) exhibited a significant (p<0.0001) decrease of dynein at the Golgi apparatus in comparison to wild-type cells (N=21). Uniquely, the R399G mutation also caused a significant and inherent fragmentation of the Golgi apparatus, which correlated with the zygosity of the mutation (+/R399G p<0.01 N=4, R399G/R399G p<0.0001 N=4). A consequent compensational response was measured as an increased interaction between the dynein intermediate chain and golgin-160 in the R399G mutant cells. Excitingly, the treatment of R399G mutant fibroblasts with tubacin (N=32), an HDAC6 inhibitor, caused a striking statistically significant (p<0.0001) amelioration of the Golgi apparatus integrity by increasing microtubule acetylation in comparison to untreated R399G mutant fibroblasts (N=33).

**Discussion and conclusions:** Using DYNC1H1 mutations, we illustrate a dynein-dependent acetylation of the microtubule network, which if aberrant and compounded by a decrease in the amount of dynein present on the Golgi membranes results in the fragmentation of the organelle. Intriguingly, α-tubulin acetylation, is significantly reduced in motor neurons harboring ALS associated mutant TUBA4A (OMIM: 191110). These data suggest a tentative link between genetic variations in DYNC1H1 and the microtubule cytoskeleton, which could contribute to aberrant tubulin modification, Golgi integrity, and axonal transport and consequently susceptibility to ALS. Importantly, we show that ameliorating the microtubule acetylation is sufficient to rescue the Golgi integrity, thereby providing a potential therapeutic target for this pathology.

**Acknowledgements:** This work was supported by Hans and Merit Rausing and Marion Brownridge

DOI: 10.1080/21678421.2016.1232060/0028

**PI58 HISTOPATHOLOGICAL FINDINGS IN AN ADULT DOWN SYNDROME PATIENT PRESENTING WITH ALS**

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**Keywords:** Down syndrome, TDP-43, SOD1

**Introduction:** A connection between Alzheimer’s disease and Down syndrome has been described extensively in the literature. The higher risk for Alzheimer’s disease in people with Down syndrome has been attributed to the increased production of amyloid beta due to an extra copy of chromosome 21, but other genes on chromosome 21 may also play a role, such as superoxide dismutase (SOD1). Little is known about the consequences of trisomy 21 for other neurodegenerative diseases.

**Case study:** We present the case of a 49-year-old male diagnosed with trisomy 21 at an early age, who developed severe intellectual deficiency and progressive muscle weakness of the upper limbs at the age of 47. He also complained of pain in the shoulders, and became unable to move his right arm within months. The symptoms worsened over the following year. He progressively stopped walking, developed dysphagia, further impairment of the lower limbs and eventually died.

**Results:** At the autopsy, the patient presented characteristic craniofacial morphological features of Down syndrome. Macroscopic examination of the brain showed atrophy of the superior temporal gyrus and the frontoparietal cortex, a small hippocampus and dilatation of the lateral ventricles. Microscopic examination of the brain showed typical features of Alzheimer’s disease with amyloid deposits in the cerebral cortex, basal ganglia, and cerebellar cortex. Gallyas staining showed the presence of numerous neuritic plaques and widespread neurofibrillary degeneration at the level of the hippocampus. Immunohistological stains for alpha-synuclein did not reveal the presence of Lewy bodies. The spinal cord examination showed atrophy of the corticospinal tract. CD68 immunohistochemistry revealed abundant macrophages in the medullary pyramids and lateral columns and an associated microglial reaction. TDP-43 immunohistochemistry showed a filamentous staining in the cytoplasm and a loss of nuclear staining within motor neurons. Ubiquitin immunohistochemistry showed weak staining of some spinal nerve roots. Interestingly, SOD1 immunohistochemistry showed a staining in the cytoplasm colocalizing with TDP-43.

**Conclusion:** The present case shows that neurodegenerative disease in Down syndrome patients can take other forms besides Alzheimer’s disease, including amyotrophic lateral sclerosis. In familial ALS (FALS), any one of the reported SOD1 inherited mutations in the SOD1 gene can lead to misfolding of the protein exerting its toxic gain of function. As cytoplasmic TDP-43 inclusions in mutant SOD1-related FALS cases are relatively rare, the presence of cytoplasmic misfolded SOD1 colocalizing with TDP43 suggests that the present case is sporadic in nature and that non-native conformers of SOD1-linked ALS and SALS might converge on a common pathogenic pathway.

DOI: 10.1080/21678421.2016.1232060/0029
P159 IBM WATSON USES ARTIFICIAL INTELLIGENCE TO SUGGEST ADDITIONAL PRION DOMAIN CONTAINING PROTEINS LINKED TO ALS

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Keywords: prion-like domain, RNA binding protein, artificial intelligence

Background: Several proteins linked to ALS and FTD contain prion-like domains that can induce protein aggregation and cell toxicity. Prion-like domains are low complexity amino acid sequences enriched in polar, uncharged amino acids. A recent study identified 240 genes in the human genome that encode for proteins greater than 60 amino acids in length containing a prion-like domain. Many of these genes encode for RNA- or DNA-binding proteins. Several RNA-binding proteins that contain prion-like domains are linked to genetic forms of ALS, including TDP-43, FUS, TAF15, hnRNPA1, and hnRNPA2B1.

Objectives: We sought to identify additional prion-like proteins associated with ALS using computational methodologies that analyze prior published information to suggest new proteins with prion-like domains with a connection to ALS. The cognitive capabilities of IBM Watson enable it to extract domain-specific text features from published literature to identify new connections between entities of interest, such as genes, proteins, drugs, and diseases. This approach has been successfully applied to gain new insights into oncology, but has not been applied to neuroscience.

Methods: We used IBM Watson to identify additional proteins containing prion-like domains that are linked to ALS. IBM Watson analyzed published abstracts to learn the text patterns of a set of known prion-like domain containing proteins linked to ALS, and then applied that learning to a candidate set of proteins ranking them by their text pattern similarity to the known prion-like domain “training set.” This training set contained 11 proteins with prion-like domains linked to ALS via genetics or associated with ALS pathology. To evaluate model performance, we used a leave-one-out cross-validation approach to determine the ranking of each training protein if it were left out of the training set. High statistical significance (p<10^-8) indicated good model performance and gave confidence in Watson’s ranking. In total, Watson ranked 221 prion-like domain containing proteins by semantic similarity to the training set.

Results: hnRNPU was the top candidate identified by IBM Watson. hnRNPU is also called Scaffold Attachment Factor A and functions in packaging hnRNA into ribonucleoprotein complexes and binds noncoding RNAs. Additional top candidates included hnRNPH1 (hnRNPA2B1 binding protein), TIAL1, and Caprin 1.

Conclusions: Overall, our approach using IBM Watson to mine scientific literature to find new prion domain-containing proteins linked to ALS has identified another such protein that exhibits abnormalities in ALS and additional prion-domain containing proteins that may provide novel insights into protein-level changes during progression of ALS and other neurodegenerative diseases.

Acknowledgements: Funding support for this project provided by the Barrow Neurological Foundation to RB.

DOI: 10.1080/21678421.2016.1232060/0030

P160 MECHANISMS OF TOXICITY OF CYANOBACTERIAL TOXINS IMPLICATED IN SPORADIC ALS

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Keywords: cyanotoxins, sporadic MND, proteomics

Background: The high incidence of ALS-Parkinsonism-Dementia complex reported on Guam in the 1950s was linked to exposure to cyanobacterial toxins. Epidemiological studies in USA, France, and Sweden support the view that exposure to cyanobacterial toxins increases the risk of developing ALS. Biomagnification of the cyanobacterial toxin β-methylamino-L-alanine (BMAA) through human food chains (molluscs and fish) has also been reported. Most cyanobacteria synthesize both of the non-protein amino acids (NPAAs) BMAA and 2,4 diaminobutyric acid (DAB). Little is known, however, about the toxicity of DAB and the toxicity of BMAA and DAB in combination. NPAAs can be neurotoxic due to their ability to mimic/replace protein amino acids in metabolic pathways, become mistakenly incorporated into proteins or to exert excitotoxic effects on glutamate receptors. Although multiple mechanisms of toxicity have been proposed for BMAA no one has carried out systematic analysis to determine how BMAA affects the whole proteome.

Objectives: (i) To examine the ability of DAB to enter the human food chain; (ii) To use proteomic approaches to examine the range of mechanisms by which BMAA and DAB could initiate neurodegenerative disease.

Methods: We carried out proteomic analysis on human neuroblastoma cells treated with BMAA (500μM) and DAB (500μM), and BMAA and DAB in combination to determine which cellular pathways were altered.
We treated *M. sativa* (alfalfa) with a range of concentrations BMAA and DAB and examined their uptake and distribution.

**Results:** DAB and BMAA were both taken up by *M. sativa* and using HPLC and triple quadrupole MS/MS we demonstrated that they were associated with plant proteins. On treatment of neuroblastoma cells with BMAA and DAB, 200–300 proteins were differentially expressed. Mitochondria were targeted as well as proteins associated with ER and proteotoxic stress. Network analysis revealed significant changes in proteins grouped as “neuromuscular disease,” “movement disorders,” and “disorders of basal ganglia.” These data are currently being investigated further by western blotting and RT-qPCR.

**Discussion:** The NPAAs BMAA and DAB are both synthesized by cyanobacteria, coexist in nature and have the potential to enter the human food chain. Proteomic analysis revealed changes in a range of cellular pathways clearly linked to human neurological disease. Exposure to both BMAA and DAB had an additive effect. As demonstrated recently in a vervet study (1) BMAA can cause neurofibrillary tangles (NFT) and β-amyloid plaque-like deposits in the brain; however, the complete spectrum of BMAA neurotoxicity and that of DAB are not fully understood.

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**DOI:** 10.1080/21678421.2016.1232060/0031

**P161 A MULTIDISCIPLINARY STUDY OF SALS IN PATIENTS ORIGINATING FROM A RESTRICTED GEOGRAPHICAL AREA**

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**Keywords:** metals, proteomics, multidisciplinary study

**Background:** Genetic and environmental factors are involved in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) and metals metabolism has been linked to ALS (1). Proteomic studies are currently being performed to search for possible biomarkers (2). Here, we present a study aimed at investigating different aspects of the disease, based on a multidisciplinary approach. The cohort of ALS patients that we analyzed includes seven patients, all originating from a common, restricted, geographical area, and five matched controls. Environmental exposure is the same for all these subjects.

**Methods:** SOD1, FUS, TDP43, C9ORF72, and APOE genotypes were evaluated. For metal quantitation, samples of serum and whole blood were analyzed by ICP-MS. For proteomic analyses, immobilized pH gradient covered the 4–10 and 3–7 pH range both in reducing and nonreducing conditions. Levels of DNA oxidation were evaluated by a comet assay. Statistical analyses were carried out with Student’s t-test and Artificial Neural Networks.

**Results:** Among the metals analyzed in serum, as concentration resulted significantly lower in patients than in controls (*p*<0.007); Mn and Hg showed lower levels in patients. Auto-CM analysis linked closely high concentrations of Al and Se to the ALS group. Levels of metals in whole blood have been correlated with levels in serum. Our proteomics data show that some proteins related to Acute Phase Response (APR) and lipid homeostasis are decreased in patients (APOA1, APOA2, TTR, RET4, and SAP) while only ANT3 results increased. For some of these proteins we can describe a drastic reduction in the first 5 years of disease. APOE4 allele is more represented in the patient’s group than in controls.

**Discussion:** Impaired metal homeostasis, attributable to environmental exposure, could lead to mineral overload. Waters of the creek of the narrow valley, where these subjects are located, are reported to be strongly polluted due to acid mine drainage. Besides promoting oxidative stress, metals can compete for the binding sites of metal-containing proteins, such as those containing iron-sulfur clusters (3). The different expression of the APR proteins reported could be a reflection of the disease status of the subjects analyzed, possibly linking ALS to a chronic inflammation status. Enrichment in APOE4 allele frequency in patients may provide a link between neurodegeneration and metabolic disturbances. It is important to highlight the fact that all the proteins found differentially expressed in our study have already been described in other studies. This strengthens our methodological approach, based on a small number of patients but with a common environmental exposure.

**References**

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**DOI:** 10.1080/21678421.2016.1232060/0032