POSTER SESSION 1 WEDNESDAY 17.30-19.00

BIOMARKERS:

1917: **MRI of the cervical spinal cord predicts respiratory dysfunction in ALS.**

Danel-Brunaud V(1), Grolez G (1), Kyheng M(2), Lopes R(3), Moreau C(1), Timmerman K(4), Auger F(5), Kuchcinski G (3), Duhamel A(2), Jissendi-Tchofo (3,6), Besson P(3), Laloux C (4), Petrault M(4), Devedjian JC(4), Rolland AS (4), Perez T(7), Pradat PF(8), Defebvre L(1), Bordet R(4), Devos D(1,4).
(1) Département de Neurologie, Université de Lille, CHU de Lille, INSERM UMRS_1171, LICEND COEN Center, Lille, France
(2) Département de Biostastistiques, Université de Lille, CHU de Lille, Lille, France
(3) Service de Neuroradiologie, Université de Lille, CHU de Lille, INSERM UMRS_1171, LICEND COEN Center Lille, Lille, France
(4) Service de Pharmacologie, Médicale Université de Lille, CHU de Lille, INSERM UMRS_1171, LICEND COEN Center Lille, Lille, France
(5) Plateau d'imagerie préclinique, Université de Lille, CHU de Lille, Lille, France
(6) Department of Radiology, Neuroradiology section, Free University of Brussels, CHU Saint-Pierre, Brussels, Belgium
(7) Service de Pneumologie, Université de Lille, CHU de Lille, Lille, France
(8) Laboratoire d'Imagerie Biomédicale, CNRS, INSERM, Sorbonne Universités, UPMC Univ Paris 06 & Département de Neurologie, Centre référent SLA, APHP, Hôpital Pitié-Salpêtrière, Paris, France

For patients with amyotrophic lateral sclerosis (ALS), the primary therapeutic goal is to minimize morbidity. Non-invasive ventilation improves survival. We aim to assess whether Magnetic Resonance Imaging (MRI) of the cervical spinal cord predicts the progression of respiratory disorders in ALS. Brain and spinal MRI was repeatedly performed in the SOD1G86R mouse model, in 40 patients and in healthy controls. Atrophy, iron overload, white matter diffusivity and neuronal loss were assessed. In Superoxide Dismutase-1 (SOD1) mice, iron accumulation appeared in the cervical spinal cord at symptom onset but disappeared with disease progression (after the onset of atrophy). In ALS patients, the volumes of the motor cortex and the medulla oblongata were already abnormally low at the time of diagnosis. Baseline diffusivity in the internal capsule was predictive of functional handicap. The decrease in cervical spinal cord volume from diagnosis to 3 months was predictive of the change in slow vital capacity at 12 months. MRI revealed marked abnormalities at the time of ALS diagnosis. Early atrophy of the cervical spinal cord may predict the progression of respiratory disorders, and so may be of value in patient care and as a primary endpoint in pilot neuroprotection studies.

1919: **Motor reorganisation in amyotrophic lateral sclerosis: coexisting degenerative and compensatory changes.**

Malek Abidi (1), Giovanni de Marco (1), Annabelle Couillandre (1)(5), Maryse Feron (1), Eya Mseddi(1), Nicolas Termoz (1)(3), Peter Bede (2)(3)(4), Pierre-François Pradat (2)(3) .
(1) Laboratoire CeRSM (EA-2931), UPL. Université Paris Nanterre, F92000 Nanterre, France.
(2) Département de Neurologie, Centre Référent Maladie Rare SLA, Hôpital de la Pitié-Salpétrière, Paris, France.
(3) Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France.
(4) Computational Neuroimaging Group, Trinity College, Dublin, Ireland.
(5) COMUE Université Paris Lumières.

Although motor neuron loss is the hallmark feature of ALS, the functional reorganisation of motor control in face of relentless motor neuron degeneration has not been comprehensively characterised. The majority of functional studies in ALS focus on motor execution alone and

little attention has been paid to movement preparation and movement initiation to date. In order to examine adaptive processes in the face of motor cortex degeneration, we performed a prospective functional neuroimaging study to characterise changes in connectivity patterns. Seventeen ALS patients with lower motor neuron predominant (LMNp) disability, thirteen patients with upper motor neurons predominant (UMNp) disease and fourteen healthy controls performed a self-initiated and coordinated motor task during fMRI. First a whole-brain analysis was performed to compare activation patterns between LMMp, UMNp, and healthy controls during the motor task. Subsequently, additional hypothesis-driven analyses were carried out using the supplementary motor area (SMA), cerebellum and striatum as seed regions. Increased cerebellar and decreased dorsolateral prefrontal cortex (DLPFC) and premotor areas (SMA) activation was identified in UMNp patients compared to healthy controls during self-initiated movement. Increased cerebellar activation was also detected in UMNp patients compared to LMNp patients. Compared to healthy controls, UMNp patients exhibit increased effective connectivity between the cerebellum and the caudate (pFWE=0.02) and decreased effective connectivity between premotor area and the caudate (pFWE=0.01), and between premotor area and cerebellum (pFWE=0.04) when performing a self-initiated movement. Similarly, compared to LMNps, significantly enhanced effective connectivity between cerebellum and caudate (pFWE= 0.01) and decreased connectivity between premotor and cerebellum (pFWE=0.01) was also recorded in UMNps. Our findings indicate that despite the dysfunction of premotor-striatal and premotor-cerebellar circuitry, cerebello-striatal connectivity increases in UMNp suggestive of compensatory or adaptive processes. Our results confirm that the clinical symptoms of ALS are the manifestation of coexisting neurodegenerative and attempted adaptive changes and circuits with increased and decreased connectivity contribute to the heterogeneity of symptoms. Our findings also highlight the rationale for targeted rehabilitation efforts in ALS to support compensatory processes.

1924: **SPiQE: an automated analytical tool for detecting and characterising fasciculations in amyotrophic lateral sclerosis.**

James Bashford* (1), Aidan Wickham (2), Raquel Iniesta (3), Manos Drakakis (2), Martyn Boutelle (2), Kerry Mills (1), Chris Shaw (1).

1 UK Dementia Research Institute, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK.

2 Department of Bioengineering, Imperial College London, UK.

3 Department of Biostatistics and Health Informatics, King's College London, UK.

Objectives: Fasciculations are a clinical hallmark of amyotrophic lateral sclerosis (ALS). Compared to concentric needle EMG, high-density surface EMG (HDSEMG) is non-invasive and records fasciculation potentials (FPs) from greater muscle volumes over longer durations. To detect and characterise FPs from vast data sets generated by serial HDSEMG, we developed an automated analytical tool.

Methods: Six ALS patients and two control patients (one with benign fasciculation syndrome and one with multifocal motor neuropathy) underwent 30-minute HDSEMG from biceps and gastrocnemius monthly. In MATLAB we developed a novel, innovative method to identify FPs amidst fluctuating noise levels. One hundred repeats of 5-fold cross validation estimated the model's predictive ability.

Results: By applying this method, we identified 5,318 FPs from 80 minutes of recordings with a sensitivity of 83.6% (+/- 0.2 SEM), specificity of 91.6% (+/- 0.1 SEM) and classification accuracy of 87.9% (+/- 0.1 SEM). An amplitude exclusion threshold (100_V) removed excessively noisy data without compromising sensitivity. The resulting automated FP counts were not significantly different to the manual counts (p=0.394).

Conclusion: We have devised and internally validated an automated method to accurately identify FPs from HDSEMG, a technique we have named Surface Potential Quantification Engine (SPiQE). Longitudinal quantification of fasciculations in ALS could provide unique insight into motor neuron health.

1944**: Characterization of blood-derived macrophages in familial and sporadic forms of Amyotrophic Lateral Sclerosis (ALS).**

Matthieu Ribon*(1), Adèle Hesters(1)(2), Aude Chiot (1), Maria del Mar Amador(2), Gaëlle Bruneteau (2), Timothée Lenglet (2), Philippe Couratier (3), Stéphanie Millecamps (1), Delphine Bohl (1), Christian S Lobsiger. (1), François Salachas (1)(2), Séverine Boillée (1).
(1) Institut du Cerveau et de la Moelle épinière, ICM, Inserm U 1127, CNRS UMR 7225, Sorbonne Université, F-75013, Paris, France. S.Boillée Team, ALS causes and mechanisms of motor neuron degeneration.
(2) Centre de référence Maladies rares SLA-IDF, Département de Neurologie, Hôpital Universitaire Pitié-Salpêtrière, Paris, France.
(3) Service de Neurologie, Centre de ressources et de compétences SLA, CHU Dupuytren, Limoges, France.

Although motor neurons (MNs) are the cells degenerating in ALS, other cell types surrounding the MNs have been shown to participate to the ongoing neurodegeneration. More specifically, previous work from our team showed a deleterious role of microglial cells, the macrophages of the central nervous system, in disease progression. Our hypothesis is that, at the periphery, monocytes/macrophages could be involved in an analogous detrimental mechanism since macrophages are in direct contact with spinal cord MN axons. ALS monocytes/macrophages could show specific reactive profiles both through their expression of ALS-linked genes and their reaction to MN degeneration. In addition, macrophages at the periphery would be an easier target for therapy than microglia in the CNS. Our aim is therefore to characterize the reactive profiles of blood monocyte and monocyte-derived macrophage populations activated with different stimuli, obtained from ALS patients with familial (FALS) or sporadic (SALS) forms and healthy controls.
To determine macrophages involvement in the disease associated with potential dysfunctions of those cells, we studied monocytes/macrophages subpopulations from 18 patients carrying disease associated mutations or SALS cases and 8 controls. Monocytes from whole blood were differentiated in vitro into macrophages and then activated with pro- or anti-inflammatory stimuli. Immunological responses of monocytes/macrophages were studied at different levels: transcriptome, cells surface marker expression, and secretion profile.
Preliminary results demonstrate that, as monocytes from healthy controls, monocytes from ALS patients were able to differentiate into macrophages, and that they could be activated into pro-inflammatory or anti-inflammatory phenotypes. We then compared FALS derived macrophages with SALS macrophages and control macrophages, using three different activation conditions. Transcriptome analysis showed that our samples were clustering in response to the stimuli. Further analyses of our transcriptomics data are now needed to detect potential defects linked to ALS pathology. Flow cytometry analyses showed that macrophages differentiated from mutant SOD1 and C9orf72 monocytes expressed less anti-inflammatory markers on their surface, which needs to be confirmed on a larger population.

1954: **Nuclear lipidome is altered in amyotrophic lateral sclerosis: a preliminary study.**

Omar Ramírez (1), Mariona Jové (1), Pascual Torres (1), Joaquim Sol (1), Laia Fontdevila(*) (1), Ricardo Romero-Guevara (1), Victòria Ayala (1), Chiara Rossi (1), Jordi Boada (1), Mònica Povedano (2), Isidro Ferrer (3) (4), Reinald Pamplona (1), Manuel Portero-Otin (1).

(1) Departament de Medicina Experimental, Facultat de Medicina, IRBLLEIDA-UDL, Avda Rovira Roure, 44, 25008, Lleida, Spain.
(2) Servei de Neurologia, Hospital Universitari de Bellvitge, Universitat de Barcelona, L'Hospitalet de Llobregat, c/La Feixa Llarga, S/N 08908 Hospitalet de Llobregat, Barcelona, Spain.
(3) Institut de Neuropatologia, Hospital Universitari de Bellvitge - IDIBELL, Universitat de Barcelona, Spain.
(4) CIBERNED (Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas), Instituto Carlos III, Spanish Ministry of Health, Spain. L'Hospitalet de Llobregat, c/La Feixa Llarga, S/N 08908 Hospitalet de Llobregat, Barcelona, Spain.

Nuclear transport functions are altered in amyotrophic lateral sclerosis (ALS). Since this may depend on the functional integrity of nuclear envelope, we wanted to ascertain whether lipid composition is affected by ALS status. In this pilot study, we isolated nuclei from frozen necropsy samples from lumbar spinal cord in sporadic ALS patients (n=4) and from healthy, age and gender matched individuals (n=4). We performed a non-targeted lipidomic analyses using liquid chromatography coupled to time-of-flight mass spectrometry. The results demonstrate that nuclei in spinal cord nuclei exhibit a differential lipidomic signature, which is able to generate a partial-least-square discriminant analyses model with a 95% of accuracy. Among the differential lipid species (n=151, p ranging between 9.46x10E-15 to 0.05 in Students T test), we could annotate 39 potential identities. These comprise several typical membrane-bound lipids such as phosphatidylethanolamines – including plasmalogens- and phosphatidylcholines but also other lipid classes such as glycosphingolipids, diacylglycerols and triacylglycerides (potentially present as nuclear lipid droplets). These results were orthogonally validated by immunohistochemistry and western-blot analyses, showing loss of alkyldihydroxyacetonephosphate synthase (AGPS), a key peroxisomal enzyme in plasmalogen synthesis, both in ALS samples and in hSODG93A transgenic mice. Further, diacylglycerol content changes were associated to ALS-linked variations in related-enzymes, such as phospholipase C ßI (PLCßI), the source of nuclear diacylglycerol, and protein kinase CßII (PKCßII), whose function partially depends on nuclei concentration of diacylglycerol. All in all, these results point out for not only an important effect in membrane lipids, but also to lipids present in nucleoplasm, suggesting an undisclosed role for this part of the subcellular lipidome in ALS pathophysiology.

1962: **Gene expression biomarkers in ALS and FTD patients: a cross-sectional study in lymphocytes**.

Gabriel García-Salamero (1), Daniel Borrego-Hernández (1), Ana Cristina Calvo (2), María del Carmen Herrero-Manso (3), Pilar Cordero-Vázquez (1), Alberto Villarejo-Galende (4), Sara Llamas-Velasco (4), Marta González-Sánchez (4), Miguel Ángel Martín-Casanueva (5), Jesús Esteban-Pérez (1), Rosario Osta (2) and Alberto García-Redondo (1).
1 Neurology department – ALS Unit. CIBERER. Health research Institute, Hospital Universitario 12 de Octubre – Avda. Córdoba s/n – 28041 Madrid – Spain.
2 LAGENBIO-I3A, Aragonese Institute of Health Sciences (IACS), Faculty of Veterinary, University of Zaragoza – Miguel Servet, 177 – 50013 Zaragoza – Spain.
3 Rheumatology department – Health Research Institute, Hospital 12 de Octubre – Avda. Córdoba s/n – 28041 Madrid – Spain.
4 Neurology department – Dementia Unit. CIBERNED. Health research Institute, Hospital Universitario 12 de Octubre – Avda. Córdoba s/n – 28041 Madrid – Spain.
5 Biochemistry department. CIBERER U-723. Health research Institute, Hospital 12 de Octubre – Avda. Córdoba s/n – 28041 Madrid – Spain.

Background: Previous studies performed on muscle biopsies from mice SOD1G93A suggested that this animal model presents an alteration in the expression of five genes (Mef2c, Gsr, Col19a, Calm1 and Snx10). In this point, we studied the expression level of eight genes (Gsr, Col19a, Mef2c, Snx10, Gsk3, Impa1, Sod1 and Nogoa) in lymphocytes of

ALS and FTD patients. These genes are involved in several metabolic pathways related with the physiopathological causes [1,2,3,4].
The aim of this study is to determinate the diagnostic capacity of these potential biomarkers both on ALS and FTD, and their clinical variants.
Methods: cDNA serial samples from lymphocytes of 45 patients with ALS (27 males and 18 females) and 58 patients with FTD (33 males and 25 females) were subjected to qPCR in order to study expression levels of the genes mentioned above. These levels were related to the main clinical parameters like days since onset of symptoms, clinical variant and others (ALSFRS-r, ALSFRS-r slope, diagnostic delay, age of onset of symptoms…). Statistical analyses were carried out with SPSS statistical software support.
Results: As regards to the ALS group, significant differences were found between patients and controls in the expression of NOGOA, IMPA1 and GSR. Into expression of GSR, two sub-groups were significantly differentiated, which were correlated with sex. Moreover, COL19A1 expression correlated significantly with inheritance (familial/sporadic).
Respecting the FTD group, significant differences were found between patients and controls in the expression of GSK3, SOD1, NOGOA and SNX10. In this case, the expression rates of these genes improved the differences between patients and controls. Besides, IMPA1 and GSR were significantly correlated with the FTD´s clinical variant.
Discussion: The expression of genes with significant differences between ALS and FTD groups and control group could perform as good diagnostic biomarkers. Specially, IMPA1 and GSR could improve the diagnosis of the clinical variant in FTD patients. More studies in other centers and with larger cohorts are necessary to improve the biomarkers sensibility and reliability to validate them as proper diagnostic/prognostic biomarkers for these diseases.

1980: **Cortico-Muscular Coherence Patterns in Motor Neuron Disease.**

Amina Coffey(1)*, Teresa Buxo (1), Antonio Fasano (1,2), Stefan Dukic (1), Mark Heverin (1), Madeleine Lowery (3), Richard Carson (4), Bahman Nasseroleslami (1), Orla Hardiman (1,5).
1. Academic Unit of Neurology, Trinity College, Dublin, Ireland.
2. Department of Neuroscience, Azienda Ospedaliero-Universitaria di Modena, University of Modena and Reggio Emilia, Modena, Italy.
3. School of Electrical & Electronic Engineering, UCD, Dublin, Ireland.
4. Trinity College Institute of Neuroscience, Trinity College, Dublin, Ireland.
5. Beaumont Hospital, Dublin, Ireland.

Background: Neurodegenerative conditions such as MND/ALS are associated with widespread changes in the motor networks. There is preliminary evidence from (f)MRI studies that changes beyond the primary motor cortices in frontal and parietal cortices may reflect direct impairment or compensatory over activity of cortical and spinal networks. The recording of joint multi-channel electroencephalogram (EEG) and electromyogram (EMG) for time-series analysis can reflect the communication between cortical brain regions by quantifying the oscillatory motor drives to muscles during specified motor tasks; hence, providing direct neuro-electric signatures of network disruption.
We hypothesize that cortico-muscular coherence (CMC) between EEG-EMG can interrogate disease-specific alterations in the brain's motor networks within and beyond the primary motor cortex in MND.
Objectives: To study CMC as a potential tool for assessing network disruption in selected motor subsystems in MND patient subgroups during functional isometric motor tasks.
Methods: High-density 128-channel EEG and 8 bipolar surface EMG recordings from extrinsic and intrinsic hand muscles were obtained from 9 patients with dominant upper (primary lateral sclerosis), 10 lower (Poliomyelitis) and 7 mixed upper/lower (amyotrophic

lateral sclerosis) motor neuron degeneration as well as on 11 healthy controls, during isometric precision grip tasks.
Results: Preliminary analysis of the PLS and PPS patient groups, indicate a pathological increase of cortico-muscular coherence over frontal and parietal brain regions. These include an alpha-band increase over frontal (Fz) regions (PLS) as well as abnormal coherence patterns between APB/FPB muscles and the frontal region in the PPS group.
Discussion: These preliminary results suggest that the EEG-EMG coherence during functional motor tasks mark pathological changes in the central-peripheral communication. This most likely reflects the disrupted balance of activity in the _-motor system in the primary motor cortex and other cortical projections (e.g. the _-system), as well as other pathways in the spinal cord; which in this case, appears as abnormal CMC patterns in regions other than the primary motor cortex (reflecting a possible compensation for loss of normal M1 corticospinal projections).
These changes can be potential biomarkers of the disease phenotype, as well as prospective tools for patient stratifications in the clinical settings and trials.

1989: **Serum neurofilament measurement in the specialised ALS diagnostic referral clinic.**

Josephine Robertson* (1), Elizabeth Gray (1), Alexander G. Thompson (1), Emily Feneberg (1), Kevin Talbot (1), Martin R. Turner (1).
1: Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, U.K.

Elevated serum neurofilament level is a leading prognostic and pharmacodynamic biomarker candidate in ALS and other neurodegenerative disorders. A diagnostic role in ALS is uncertain as many cases presenting to the neurologist are diagnosed with certainty on clinical grounds alone. Furthermore, neurofilament levels are inconsistently elevated in the lower motor neuron (LMN)-predominant forms of ALS that often present the greatest diagnostic challenge. To assess the 'real world' value of this measurement, we piloted a prospective study design in a specialised ALS diagnostic referral clinic.
All 22 individuals attending the Oxford University Hospitals' 'Motor Nerve Disorders' clinic over a two-month period were offered study participation in the waiting area prior to clinical assessment. Consent was obtained to take serum as part of a generic biomarker study in neurological disorders, without specifying ALS, and with no feedback of result.
One individual declined participation. Serum from 21 participants was tested for phosphorylated neurofilament heavy chain (pNfH) using a commercial ELISA kit (Euroimmun, Germany) and samples assayed in duplicate. Prior to assay, participants were grouped on the basis of the ALS neurologist's diagnosis following clinical assessment: 'Certain ALS' (n=10), 'Alternative Diagnosis' (n=6) and 'Uncertain Diagnosis' (n=5). Cutoff values were defined using Receiver Operator Characteristic analysis of serum pNfH levels in an independent cohort of patients with ALS (n=72), 'mimic' disorders (e.g. Kennedy's; n=12) and healthy age-similar controls (n=27).
Serum pNfH level effectively distinguished those diagnosed with 'Certain ALS' from all those with an 'Alternative Diagnosis'. In those labelled 'Uncertain Diagnosis', serum pNfH levels were not consistently elevated in those who subsequently received a label of ALS in clinical follow-up (detailed phenotypic data will be presented).
With this pilot study as a template, a multi-centre international study based in specialised ALS referral clinics is entirely feasible. It must focus on cases where the diagnosis is not clear on clinical grounds to an experienced neurologist and will strengthen ongoing efforts to standardise serum neurofilament assay parameters for wider prognostic and pharmacodynamic biomarker roles.

2010: **Cortical thinning associated with advancing disease stages in ALS cognitive phenotypes.**

Monica Consonni (1), Eleonora Dalla Bella (1), Valeria E. Contarino (2), Enrica Bersano (1), Giuseppe Lauria (1,3).
(1) 3rd Neurology Unit and Motor Neuron Diseases Centre, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.
(2) Department of Neuroradiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
(3) Department of Biomedical and Clinical Sciences Luigi Sacco , University of Milan, Milan, Italy.

Background: Amyotrophic lateral sclerosis (ALS) can be associated with a spectrum of cognitive and behavioural symptoms becoming more frequent with more severe disease stage. Although distinctive patterns of focal cortical atrophy have been identified in cognitively-impaired patients (ALSimp), the relations between disease progression and cortical involvement of ALSimp has been poorly investigated.
Objectives: To investigate the progression of cortical thinning across disease stages and cognitive/behavioural impairment in ALS.
Methods: Seventeen patients with mild cognitive/behavioural impairment (ALSimp), 19 cognitively-normal ALS patients (ALScn) and 26 healthy control (HC) subjects underwent a structural 3T MRI. Cortical thickness (CT) was measured with a region-wise approach in 74 bilateral brain regions. Age-corrected and z standardised CT scores (CTz) were calculated. Five levels of cortical thinning, ranging from 0 ("normal-like"; CTz > 50th percentile of the HC group) to 4 ("pathological"; CTz ≤5th percentile of the HC group), were defined for each region. The King's Clinical Staging System was used to determine disease stages. The Jonckheere-Terpstra test was performed, separately for ALSimp and ALScn groups, to test for trends in cortical thinning across disease stages (1, 2, and 3) and HC (coded "0").
Results: In ALSimp patients, the medians of CT levels of the middle frontal sulcus, the inferior temporal sulcus, the anterior cingular cortex and the lateral sulcus of the right hemisphere increased across disease stages. ALScn patients showed progressive cortical thinning in bilateral motor cortex, right middle frontal sulcus, bilateral parietal and occipital regions, left planum temporale, left lateral sulcus and right subcallosal and posterior cingular cortex.
Discussion: We attempted to study, in vivo, the anatomical counterpart of the spreading of the disease in ALS patients subdivided according to the presence of cognitive and/or behavioural impairment. We documented different patterns of progressive cortical involvement in ALScn and ALSimp patients. As disease progress, ALScn patients manifested motor and extra-motor involvement according to the neuropathological studies of disease dissemination. Conversely, ALSimp patients showed specific right hemisphere involvement embracing extra-motor fronto-insular and temporal regions.
Conclusion: ALSimp may have a specific pathological course distinct to that of the classic ALS.

2018 **Plasma neurofilament light chain for the diagnosis of neurogenerative disease.**

Ahmad Al Khleifat*(1), Nicholas Ashton(1,2,3,4), Shorena Janelidze, , Alberto Lleo, Lucilla Parnetti, (Milan-EMIF), Jan Lycke, Latha Velayudhan, Angela Hodges, Andre Strydom(3), Gil Rabinovici10, Bruce Miller(10), Pedro Rosa-Neto, Tharick Ali Pascoal, Per Svenningsson3,11, Holly Green(3,11), Carmine Parinate(12), Gorka Fernández-Eulate (13), Francisco Gil-Bea (13), Adolfo López de Muna (13), Madhav Thambisetty(14), Erik Stomrud, Alexander Santillo, Sebastian Palmqvist, Michael Schöll(1,9,15), Dag Aarsland(3,4,5), Kaj Blennow(1,8), Henrik Zetterberg(1,7,8,9) , Abdul Hye(1), Oskar Hansson (15) and Amar Al-Chalabi(1,4).
1 King's College London, Institute of Psychiatry, Psychology & Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK

2 Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden
3 Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
4 NIHR Biomedical Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS Foundation, London, UK
5 Centre for Age-Related Medicine, Stavanger University Hospital, Stavanger, Norway
6 Department of Psychiatry, University of Oxford. Warneford Hospital, Oxford, UK
7 Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK
8 Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
9 Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London UK
10 Department of Neurology, University of California, San Francisco, San Francisco, California, United States of America
11 Department of Clinical Neuroscience and Neurology, Karolinska Institutet, 17177 Stockholm, Sweden
12 Section and Laboratory of Stress, Psychiatry and Immunology (SPI-Lab), Institute of Psychiatry, King's College London, London, UK
13 Neurology department. Donostia University Hospital. San Sebastian. Spain
14 Clinical and Translational Neuroscience Unit, Laboratory of Behavioral Neuroscience, National Institute on Aging (NIA), National Institutes of Health (NIH), Baltimore, Maryland, United States of America
15 Clinical Memory Research Unit, Department of Clinical Sciences, Malmö, Lund University, Lund, Sweden.

Background: There is a need for biomarkers for neurodegenerative diseases to support the diagnosis of the disease, and to predict disease progression. Typical neurodegenerative protein markers (Amyloid tau and _-synuclein) can be readily measured in plasma but historically the correlation with disease and/or CSF measures has been absent or weak. However, the correlation is very tight between plasma and CSF neurofilament light (NfL) protein (r2 = 0.8), another axonal neuron-specific protein. The SIMOA assay for NfL has a 125-fold better analytical sensitivity than when the same anti-NFL antibodies used in other immunoassays-based platforms (Meso Scale Diagnostics [MSD] or ELISA). This is significant because NFL can now be accurately measured in blood samples from normal individuals, which are below the level for accurate quantification when using other methods. Using the SIMOA NFL assay, a recent study showed a marked increase in serum NFL in people with Amyotrophic Lateral Sclerosis (ALS) as compared with controls. Furthermore, plasma NFL has been shown to be increased in several other neurodegenerative diseases as well as increased CSF NFL in more diverse diseases. Yet, to date, there has been no study that compares the levels plasma NfL across several neurodegenerative diseases within the same study.
Methods: Plasma samples were obtained from multiple sources covering the range of neurodegenerative conditions. These included conditions such as Alzheimer's disease (n=89), frontotemporal dementia (n=49), Lewy Body Dementia (n=139), Parkinson's disease (n=64), Down Syndrome disease (DS) (n=44), Ds with dementia (n=12), ALS (n=50), and controls (n=199). A replication cohort (BioFINDER, Sweden) included the same clinical groups (n=1452).
Plasma NfL concentration was measured using the SiMOA platform (NF-light; Quanterix, Lexington, MA) at the Maurice Wohl Clinical Neuroscience Institute, London, UK. Samples were randomised, blinded and measured in duplicate using a batch of reagents from the same lot. The intra-assay and inter-assay coefficients of variance were 4.8% and 10.5% respectively. The limit of detection (LOD) was 0.52_pg/mL and the lower limit of quantification (LLOQ) was 3.26_pg/mL when compensated for a 4-fold sample dilution.
Results: Highest was observed in ALS (143.9 pg/mL), ALS-FTD (100 pg/mL) and DLB/PDD (79.6 pg/mL) individuals. However, association were observed in 5 neurodegenerative conditions (ALS, ALS-FTD, CBS, DS-D, DLB,PDD).

2022: **A prospective multicenter French study of brain and spinal cord imaging in ALS patients.**

Pierre-Francois Pradat (1, 2, 3), Anne Sophie Rolland (4), Gregory Kuchinski (5), Renaud Lopes (5), Nacim Betrouni (5), Jean Pierre Pruvo (6), Giorgia Querin (1,2), Chupin Marie (7), Gay David (7), El Mendili Mounir (2,8), Djobeir Sonia (7), David Devos (3, 9) for the PULSE study group.
1- APHP, Département de Neurologie, Centre Référent SLA, Hôpital Pitié-Salpêtrière, Paris, France.
2- Laboratoire d'Imagerie Biomédicale, CNRS, INSERM, Sorbonne Université, Paris, France.
3- Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute Ulster University, C-TRIC, Altnagelvin Hospital, Londonderry, United Kingdom.
4- Department of Medical Pharmacology, Lille University, INSERM UMRS_1171, University Hospital Center, LICEND COEN Center, Lille, France.
5- Université Lille, Inserm, CHU de Lille, UMR_S 1171, Degenerative and vascular cognitive disorders, Lille, France.
6- Department of Neuroradiology, Roger Salengro Hospital, CHU Lille, University of Lille, Lille, France/UMR 1171, CHU Lille, University of Lille, Lille, France.
7- Centre pour l'Acquisition et le Traitement des Images, Institut du Cerveau et de la Moelle Épinière, Paris, France.
8- Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, USA.
9- Department of Neurology, ALS Center, Lille University, INSERM UMRS_1171, University Hospital Center, LICEND COEN Center, Lille, France.

Neuroimaging in ALS has gained unprecedented momentum in recent years and has emerged as a sensitive clinical tool with true biomarker potential. For validating neuroimaging biomarkers, there is a need for large cohorts of patients with multipoint longitudinal assessment and combination of brain and spinal cord imaging.
Objectives: The objective of this study was to investigate whether brain and spinal cord MRI can provide useful biomarkers for the diagnosis, the prediction of functional decline as well as for monitoring the progression of the disease.
Methods: This longitudinal MRI study is part of a prospective observational multicenter French study: Study of Predictive Factors of Progression of Lateral Amyotrophic Sclerosis (PULSE, NCT02360891). French ALS centers (n=18) will include 1,000 ALS patients for a multimodal assessment including phenotyping, genotyping, biobanking (serum, plasma, skin and muscle biopsy), electrophysiology and neuroimaging. The neuroimaging protocol includes anatomic sequences (T1- and T2-weighted) at brain, cervical, thoracic and lombo-sacral level and T2 FLAIR-weighted sequence for the brain. Diffusion imaging is acquired at brain and cervical spinal cord level to study white matter connectivity. Resting state functional brain MRI (fMRI) is acquired to study functional network modifications. A multiecho T2*-weighted sequence is acquired in 3D to investigate R2* modifications for the brain and in 2D at the cervical level to study spinal white and grey matter atrophy. For 3T systems, sequences were standardized from clinically relevant product sequences, for systems from each MRI manufacturer and as much as possible between MRI manufacturers.
Results: In this ongoing study, 292 ALS patients, 53 healthy controls and 3 neurologic controls have been included. Longitudinal assessment has already been performed in 145 patients at Month 6, 81 at Month 12, 36 at Month 18, 19 at Month 24 and 2 at Month 36. Clinical, genotypical, biological as well as electrophysiological parameters (motor unit count by the MUNIX method and transcranial magnetic stimulation) have been collected.
Discussion: In the context of the well-recognized heterogeneity of ALS, this large multimodal prospective study will investigate neuroimaging biomarkers for diagnosis, prognosis and as progression markers useful for clinical trials.

2027: **Circulating microRNAs as biomarkers in motor neurone disease**.

Nicole Hindley1, Suresh Chhetri2, Vicky Jones1 and Lisa Shaw1.

1School of Pharmacy and Biomedical Science, University of Central Lancashire, Preston, PR1 2HE UK. Preston MND Care and Research Centre, Royal Preston Hospital, Preston, PR2 9HT UK.

MicroRNAs (miRNAs) are small RNA sequences 22-25 nucleotides in length which mediate post-transcriptional gene regulation by binding to specific target mRNA sequences to prevent their translation. Recently, miRNAs have been revealed to be regulators of many processes within the brain and their dysregulation has been implicated in various neuropathies, including ALS. MiRNAs can be released into the circulation and it has been shown that the profiles of miRNAs in the biofluids of patients of various pathologies are altered compared to healthy controls. Such altered miRNA signatures are therefore being explored as tools for the identification and classification of a number of diseases, although studies involving ALS biofluids have not yet identified a definitive diagnostic profile.
We compared miRNA expression in an initial set of six serum samples from sporadic ALS patients (recruited from The Royal Preston Hospital, UK) and six serum samples from age- and sex-matched controls using a human miRNA PCR array. Differentially expressed miRNAs were validated using qPCR on an additional patient cohort consisting of 30 serum samples from each group. Data were stratified according patient characteristics, revealing distinct profiles according to patient gender and age.
These preliminary results reveal novel dysregulated miRNAs not identified in other studies, and highlight the importance of patient stratification in the development of biomarker profiles, pointing to the requirement of a more personalised approach to diagnosis of ALS. Future work will explore these dysregulated miRNAs in more detail.

2044: **Chitinase levels in the pre- and peri-symptomatic phases of ALS**.

Elizabeth Gray (1), J oe Pelt (1), Joanne Wuu (2), Alexander G Thompson (1), Kevin Talbot (1), Michael Benatar (2), *Martin R Turner (1).
(1) Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK.
(2) Department of Neurology, University of Miami, Miami, Florida, USA.

Background: A neuroinflammatory response involving prominent microglial infiltration is a consistent feature of ALS histopathology. Primary versus secondary pathogenic roles, or disease-modifying effects of microglia in ALS remain speculative issues, and biomarkers of disease activity are an urgent priority more generally. Proteomic and ELISA studies have identified significantly elevated chitinase proteins Chitotriosidase 1 (CHIT1), chitinase-3-like protein 1 (CHI3L1, aka YKL-40) and chitinase-3-like protein 2 (CHI3L2) in the cerebrospinal fluid (CSF) of ALS patients. Asymptomatic carriers of genetic mutations associated with a high risk of developing ALS, offer an opportunity to study the inflammatory milieu during the 'compensated' period prior to the onset of symptoms.
Methods: CSF samples were obtained from individuals enrolled through 'Pre-fALS', a cohort of asymptomatic carriers of ALS-associated mutations (e.g. C9orf72, SOD1, TARDBP, FUS) assessed and sampled at regular intervals. CHIT1, CHI3L1 and CHI3L2 levels were measured by ELISA, along with CHIT1 activity in CSF taken at 1-2 year intervals from 62 initially asymptomatic individuals, with peri-symptomatic data available for 7 who phenoconverted, i.e. developed definite symptoms of ALS during follow-up. Samples from a group of 16 healthy, non-carrier, age-similar controls and from 12 symptomatic ALS patients were also studied.
Results: CSF CHIT1, CHI3L1 and CHI3L2 levels were not elevated in asymptomatic at-risk carriers compared to controls. Individual levels remained relatively stable over time. CHIT1 levels were elevated prior to phenoconversion in four of seven, and were increased by the time of phenoconversion in a further two. In five with post-conversion longitudinal data, CHIT1 levels continued to rise for at least a year. Deeper phenotype analysis and CHIT1 enzyme activity data will be presented.

Conclusions: The neuroinflammatory response, based on chitinase levels at least, appears to be a feature of the peri-symptomatic and early post-symptomatic 'decompensated' phase of ALS. The timing of the initial elevation in CSF CHIT1 relative to neurofilament elevation published in this cohort remains to be determined and will require a greater density of data from individuals captured in peri-symptomatic period. This study strengthens the place of CHIT1 as part of a growing panel of biomarkers to objectively assess the impact of future therapeutic interventions in ALS.

2045 **An fMRI study of attentional-executive function in amyotrophic lateral sclerosis.**

Xenia Kobeleva* (1, 2), Maher Zoubi (1,2), Marcel Daamen (2), Henning Boecker (1,2), Thomas Klockgether (1,2), Patrick Weydt (1, 2)
1 University Hospital Bonn, Bonn, Germany.
2 DZNE Bonn, Bonn, Germany.

Introduction: Up to 50% of ALS patients suffer from cognitive impairments (Montuschi et al., 2014; Phukan et al., 2012) due to frontotemporal neural dysfunction (Clark & Forman, 2006). These deficits complicate patients' use of assistive devices and thus have a significant impact on quality of life. In this study we aimed to characterize neural correlates of attentional-executive function in ALS using fMRI.
Methods: 14 patients with ALS and 18 matched controls performed a modified version of the attention network test in an fMRI scanner (Fan et al., 2002; Firbank et al., 2016). Subjects reacted to four arrowheads which all pointed into the same direction (congruent) or with one arrow showing into another direction (incongruent), calling for increased executive control. The level of conflict was adjusted by two incongruent task conditions.
After standard preprocessing the functional images were submitted to an analysis on a single-subject level with regressors for the onsets of each stimulus, including temporal derivatives to model variance in response latencies. Incorrect trials and motion parameters were included as covariates of no interest. Then, we performed a second-level analysis to evaluate group differences using following contrasts: executive effect (incongruent > congruent target) and conflict effect (hard incongruent > easy incongruent target), applying an uncorrected voxelwise threshold of p 0.05). During the executive contrast, both groups showed bilateral frontoparietal activations, and additional lateral occipital activations. The ALS group had a higher activation of the right insula and left frontal cortex in comparison to the control group ($p < 0.001$, uncorrected). The conflict contrast was associated with parietal activations in both groups and an increased activation of the cerebellum in the ALS group ($p < 0.001$, uncorrected).
Conclusions: Although both groups had comparable relative reaction times and error rates, we found increased activation at a trend level mostly of frontal areas during attentional-executive processing in ALS. This finding could either be caused by compensational mechanisms to overcome neuronal damage or be a sign of early brain network dysfunction that has not passed the threshold to manifest behaviorally.

2047: **A focus on disease biomarkers: from the bench towards the bedside in ALS disease.**

Ana C. Calvo (1*), Janne M. Toivonen (1), Laura Moreno (1), Miriam de la Torre (1), Leticia Moreno (1), Nora Molina (1), Raquel Manzano (1), Pilar Larrodé (1), Christian Lunetta (3), Silvana Penco (3), María Jesús Muñoz (1), Pilar Zaragoza (1), Jesús Esteban (2), Alberto García Redondo (2), Rosario Osta (1).
(1) LAGENBIO (Laboratory of Genetics and Biochemistry), Faculty of Veterinary-IIS Aragón, IA2-CITA, CIBERNED-612, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain.

(2) NEUROLOGY Department, ALS UNIT. CIBERER U-723. Health Research Institute, October 12th Hospital "IIS I+12". Avda. Córdoba s/n, 28041 Madrid, Spain.
(3) NEMO (NEuroMuscular Omnicentre) Clinical Center. Fondazione Serena Onlus. Piazza Ospedale Maggiore 3, 20162 Milan, Italy.

The search of reliable biomarkers of disease that can be accurate indicators of early symptoms, disease progression, or even patients' survival, in individuals suffering from ALS is needed. We cross-sectionally and longitudinally analyzed different biomarkers of disease, previously identified in transgenic mice, in five cohorts of ALS patients. Two main wet biomarkers were identified in skeletal muscle and blood samples. In addition, a calcium dependent biomarker was also identified cross-sectionally in post mortem samples in ALS patients. In a first cohort of 12 definite ALS patients, the miRNA-206 was found significantly and specifically elevated in serum samples from ALS patients respect to control group ($p<0.01$), suggesting that miRNA-206 upregulation could be contributing to the maintenance and regeneration of NMJ. Regarding NMJ integrity, Collagen type XIX, alpha 1 (COL19A1) is a matrix protein involved in muscle physiology and differentiation. In two cross-sectional studies performed in two different cohorts, including a total of 228 sporadic and familial ALS patients, increasing COL19A1 protein and gene levels were associated with a faster progression of the disease, showing a mortality risk of 70.5% ($p<0.05$) and 92.47% ($p<0.01$) in muscle biopsies and blood samples, respectively. In a longitudinal study enrolling 40 sporadic ALS patients monitored at 6-monthly intervals during a follow-up period of 24 months, increasing COL19A1 gene levels were associated with a faster progression during the follow-up period ($p<0.05$) and with a higher mortality risk (HR: 1.179, CI: 1.046-1.327, $p < 0.01$). Finally, the downstream regulatory element antagonist modulator (DREAM), which is a neuronal calcium-binding protein, was specifically localized by immunoassay in the cytoplasm and nucleus of motorneurons and in astrocytes in the spinal cord and frontal cortex from ALS patients, suggesting a detrimental role of DREAM in the last stages of the disease since the immunostaining of this protein was coincident with GFAP, Bax and caspase-3 detection. We provide evidence that miRNA-206 and COL19A1 upregulation could improve prognosis at earlier stages of the disease, while DREAM could shed light on the pathological signs related to the disease. These findings can support the clinical practice and They can be of help in future clinical trials, paving the way to promising and novel therapeutic target in ALS.

2051: **Systemic Inflammatory Profiles and Disease Progression in Amyotrophic Lateral Sclerosis.**

Nayana Gaur (1), Benjamin Vlad (1)*, Robert Steinbach (1), Tino Prell (1, 2), Otto W Witte (1, 2) Julian Grosskreutz (1, 2)
(1) Hans Berger Department of Neurology, Jena University Hospital, Jena, Germany.
(2) Center for Healthy Ageing, Jena University Hospital, Jena, Germany

Background: Systemic inflammation has been reported as a feature of several neurodegenerative conditions, including Amyotrophic Lateral Sclerosis (ALS). However, its role as a disease exacerbator and its contribution to functional deterioration is yet to be fully elucidated. A better understanding of how standard laboratory-measured inflammatory profiles relate to individual disease aggressiveness and the disease course covered is crucial.
Objectives: We aim to evaluate the association between routinely collected laboratory indicators of systemic inflammation and selected outcome metrics in ALS patients. Functional deterioration will be assessed using both the traditional ALSFRS-R-derived Progression Rate (PR) index and the novel D50 progression model.
Proposed Methods and Results: Incident laboratory blood data, including blood C-Reactive Protein, leukocyte count, erythrocyte sedimentation rate, total protein, and clinical

ALSFRS-R scores for a cohort of ~300 patients will be retrospectively evaluated. The D50 model will be used to stratify patients based on a) either summative disease aggressiveness or b) mathematical phases that are derived from individual relative disease courses. Iterative least-square fitting of all available ALSFRS-R scores was used yield D50 model indices Three indices are available for summative description: D50 (time taken for ALSFRS-R score to reach 24), dx (time constant of ALSFRS-R decay), and relative D50 (describing individual disease covered in reference to D50, with 0=disease onset and 0.5=time-point of halved functionality. Local descriptors of disease activity were also calculated for all patients: calculated functional loss & functional state at any given time during disease course. Correlations between these indices and laboratory data will be statistically evaluated as will correlations between the various lab values themselves.
Future Directions: The proposed work will build on the existing literature on systemic inflammation and its role in driving ALS disease activity by placing available data within a novel framework that can analyze how these values may affect clinical deterioration, regardless of when the individual patient was assessed.
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2058: **EEG-power in the motor network as a potential biomarker for disease progression in ALS**.

Antonio Fasano (1,2)°(*), Stefan Dukic (2,3)°, Amina Coffey (2)°, Teresa Buxó (2), Roisin Mc Mackin (2), Rangariroyashe Chipika (2), Mark Heverin (2), Peter Bede (4), Muthuraman Muthuraman (5), Madeleine Lowey (6), Richard Carson (7), Bahman Nasseroleslami (2), Orla Hardiman (2,8).
1. Department of Neuroscience, Azienda Ospedaliero-Universitaria di Modena, University of Modena and Reggio Emilia, Modena, Italy.
2. Academic Unit of Neurology, Trinity College Dublin, the University of Dublin, Dublin, Ireland.
3. Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands.
4. Computational Neuroimaging Group, Trinity Biomedical Sciences Institute, Trinity College Dublin, the University of Dublin, Ireland.
5. Biomedical Statistics and Multimodal Signal Processing Unit, Department of Neurology, Johannes-Gutenberg-University Hospital, Mainz, Germany.
6. School of Electrical and Electronic Engineering, University College Dublin, Dublin, Ireland.
7. Trinity College Institute of Neuroscience, Trinity College Dublin, the University of Dublin, Dublin, Ireland.
8. Beaumont Hospital, Dublin, Ireland.

°These authors contributed equally to this work.

Background: Most clinical trials in ALS have led to disappointing results raising concerns about the choice of outcome measures. While ALSFRS-R is the most used marker of disease progression, recent studies showed that it fails to satisfy rigorous measurement standards. On the other hand, EEG has proven to be a useful biomarker-candidate in other neurodegenerative disorders as it is a direct measure of neural activity. Additionally, a recent EEG study in ALS showed abnormal pattern in patients that correlated with MRI measures of motor system degeneration.

Aim: To investigate the resting-state EEG β-power in the motor network as a potential biomarker for disease progression in ALS.

Methods: In this pilot study, a 128-channels resting-state EEG was used to estimate _-power in the motor network in 10 ALS patients (5 F, mean age 63.1±12.9 yrs). Data were source-reconstructed using the LCMV beamformer and an atlas-based approach was applied to assess signals from the motor network. Estimated EEG power values were normalized by inverse normal transformation allowing for Pearson's correlation coefficient to be used.

Clinical examination was performed on the recording day: ALSFRS-R, muscular power assessment (deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, FDI, APB) and upper motor neuron signs evaluation (biceps, triceps and brachioradialis reflexes, Hoffmann sign). Lower and upper motor neuron score in the upper limbs (LMN and UMN scores) was calculated for each patient.

Results: β-power over in the motor network correlates with: ALSFRS-R ($r = -0.843$, $p = 0.002$), fine motor function sub-score($r = -0.731$, $p=0.016$), LMN score($r = -0.673$, $p=0.033$), UMN score($n=9$, $r = -0.746$, $p=0.021$ removing an outlier with very low beta power and UMN score), delta ALSFRS-R between disease onset and recording time($n=9$, $r = 0.727$, $p=0.027$ removing an outlier with a very slow progression rate).

Discussion: These data suggest that source-reconstructed β-band power may be a useful biomarker for disease progression in ALS. Since β-band activity is present within the sensorimotor cortex and it is mostly generated by pyramidal neurons within the fifth cortical layer and GABA-A receptors, these findings can be attributed to the cortical hyperexcitability observed in ALS, structural degeneration of pyramidal cells and loss of interneurons that entrain them.

2067: **Ferritin and LDL-cholesterol as biomarkers of fat-free mass loss in ALS.**

Pierre Jésus(1,2)* Hélène Blasco(3,4), Franck Patin(3,4), Salah Eddine Bakkouche(5), Stéphane Beltran(4,5), Christian R Andres(3,4), Patrick Vourc'h(3,4), Maillot F(4,6), Philippe Corcia(3,4).
1 Nutrition Unit, University Hospital of Limoges, Limoges, France.
2 INSERM, U1094, Tropical Neuroepidemiology, Limoges, France.
3 Laboratory of Biochemistry and Molecular Biology, University Hospital of Tours, Tours, France
4 INSERM, U1253, Imaging & Brain, François-Rabelais University, Tours, France
5 ALS center, University Hospital of Tours, Tours, France
6 Internal Medicine Department, University Hospital of Tours, Tours, France.

Introduction: The availability of longitudinal clinical and biological data led us to wonder whether these parameters could be used to predict disturbances in body composition during ALS progression.

Methods: Bioelectrical impedance analysis (BIA) to measure fat-free mass (FFM) as well as clinical (ALS functional rating scale revised, forced vital capacity) and biological parameters (albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, ferritin) were collected one year after diagnosis in ALS patients (T1 after diagnosis and T4 after one year). Comparisons of quantitative variables were realized with Mann-Whitney test. The correlations were evaluated by the Spearman test. Performances to predict the evolution of BIA parameters during ALS evolution were evaluated by ROC analysis.

Results: Forty-two ALS patients were enrolled for whom 26.8% had bulbar onset, and the median age was 70.7 years (62.7 – 78.8). Between T1 and T4, 61.9% of patients lost FFM with a median FFM loss of -12.6% (-18.4 – -2.6). In patients with FFM loss LDL-c decreased and ferritin increased significantly during the follow-up compared to those with FFM gain ($p=0.008$ and $p<0.0001$, respectively). Variations in FFM over one year were correlated to the variations in LDL-cholesterol ($r=0.53$, $p=0.002$) and ferritin ($r=-0.58$, $p=0.0002$). The Area Under the Curve's were 0.87 (confidence interval 95%: 0.75 – 0.99; $p=0.0002$) and 0.77 (0.61 – 0.93; $p=0.009$) for ferritin and LDL-c variation as biomarkers, respectively, of FFM decrease during follow-up. To predict FFM loss, an increase in ferritin over 9 µg/L had a sensitivity of 90.0% and a specificity of 80.0% ($p<0.0001$).

Conclusion: Ferritine evolution would allow to easily follow the FFM without BIA during ALS. In addition, an adapted nutritional treatment based on this biological parameter might slow down ALS progression.

2068 **Hypermetabolism is a deleterious prognostic factor in patients with Amyotrophic Lateral Sclerosis**.

Pierre Jésus (1, 2, 3), Philippe Fayemendy (1, 2, 3), Marie Nicol (2, 3, 4), Géraldine Lautrette (4), Huguette Sourisseau (1), Pierre Marie Preux (2, 3, 5), Jean Claude Desport (1, 2, 3), Benoit Marin (2, 3, 5), Philippe Couratier (2, 3, 4).
1 Nutrition Unit, University Hospital of Limoges, France
2 INSERM, U1094, Tropical Neuroepidemiology, School of medicine,
3 UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, CNRS FR 3503 GEIST, University of Limoges,
4 ALS center, University Hospital of Limoges, Limoges, France
5 Center of Epidemiology, Biostatistics and Research Methodology (CEBIMER), University Hospital of Limoges, Limoges, France.

Rationale: Hypermetabolism, defined as an excessive level of resting energy expenditure (REE) has been reported in 50-60% of patients with amyotrophic lateral sclerosis (ALS). The aim of this study was to investigate a large cohort of ALS patients in order to determine their nutritional, neurological and respiratory parameters and their survival according to the metabolic level.
Methods: Nutritional, neurological and respiratory assessments were prospectively recorded. Nutritional evaluation included body mass index, REE measured by indirect calorimetry (hypermetabolism if REE variation [_REE] > 10%) and fat mass (FM) using impedancemetry. Neurological evaluation included ALS phenotype at time of diagnosis, site at onset, ALSFRS-R score. Respiratory evaluation included vital capacity and SNIFF test. Survival analysis used the Kaplan-Meier method and the multivariate Cox model.
Results: 315 patients were analysed. Median age at diagnosis was 65.9 years. 55.2% of patients were hypermetabolic. According to the metabolic level (_REE 20%), patients with a _REE >20% initially had a lower FM, 29.7% vs. 32.1% in those ≤ 10% (p = 0.0054). SNIFF test tended to be lower in ALS patients with _REE > 20% (p = 0.07). During follow-up, nutritional status did not differ between the three groups, the median slope of ALSFRS-R tended to evolve more severely in patients with _REE > 20%, -1.4 points / month vs. -1.0 points / month in those ≤ 10% (p = 0.07). Overall median survival since diagnosis was 18.4 months. _REE > 20% tended to increase the risk of dying compared to ≤ 10% (HR = 1.33, p = 0.055). In multivariate analysis, an increased REE / FM ratio was independently associated with death (HR = 1.005, p = 0.001).
Conclusion: Hypermetabolism is present in more than half of ALS patients. It modifies the body composition at diagnosis, and patients with hypermetabolism > 20% have a worse prognosis.

2072 **Absence of hyperexcitability of spinal motoneurons in patients with ALS.**

Véronique MARCHAND-PAUVERT(1), Iseline PEYRE(1), Alexandra LACKMY-VALLEE(1), Giorgia QUERIN(1,2), Peter BEDE(1), Lucette LACOMBLEZ(2), Rabab DEBS (2) and Pierre-François PRADAT(1,2).
1.Sorbonne Université, Inserm, CNRS, Laboratoire d'Imagerie Biomédicale, LIB, F-75006, Paris, France.
2.Neurologie, AP-HP, Hôpital Pitié-Salpêtrière, F-75013, Paris, France.

Experimental models have primarily revealed spinal motoneuron hypoexcitability in ALS, which is contentious considering the role of glutamate-induced excitotoxicity in neurodegeneration and clinical features rather supporting hyperexcitability. This issue was further addressed in human patients by investigating changes in motor unit firing during contraction and relaxation. 22 ALS patients with subtle motor deficits and 28 controls performed tonic contractions of extensor carpi radialis, triceps brachialis, tibialis anterior

and quadriceps, to isolate low threshold unit (U1) in EMG. Then, they performed stronger contraction or tendon vibration was delivered, to recruit higher threshold unit (U2) during 10 sec. before they relaxed progressively. EMG and motor unit potential analyses suggest altered neuromuscular function in all muscles, including those with normal clinical evaluation (MRC score at 5). During the preconditioning tonic phase, U1 discharge frequency was not significantly different between groups. During recruitment, the increase in U1 frequency (ΔF-R) was comparable between groups both during contraction and tendon vibration. During derecruitment, the decrease in U1 frequency (ΔF-D) was reduced in ALS whatever the recruitment mode, particularly for ΔF-R < 6-8 Hz and in upper limb, matching with muscle weaknesses in the group. The ΔF-D reduction was more pronounced in patients with more functional loss and more rapid disease progression rate. This in vivo study has demonstrated lower motoneuron capacities to self-sustained discharge, related to disease progression and functional loss, and further supports that motoneurons are normo- to hypoexcitable in ALS patients, similarly to the observations in experimental models.

2075 **Cardiac troponins as biomarkers in amyotrophic lateral sclerosis.**

Castro-Gomez, Sergio(*)(1), Radermacher, Barbara(1), Widmann, Catherine(1), Binder, Julius(1), Tacik, Pawel(1), Heneka, Michael(1,2), Weydt, Patrick (1).
(1) Department of Neurodegenerative Disease and Geriatric Psychiatry, University Hospital Bonn, Bonn, Germany.
(2) German Center for Neurodegenerative Diseases, Bonn, Germany.

Troponins (Tn) are essential structural and functional components of skeletal and cardiac muscles. Elevation of cardiac isoforms of troponin I (cTnI) and T (cTnT) in serum are among the most sensitive indicators of myocardial injury and key elements in the diagnostic work-up of acute coronary syndrome. Despite the immense utility and importance of these biomarkers in day-to-day clinical practice, chronic cardiac and non-cardiac conditions are associated with elevated cardiac troponins and can lead to unnecessary diagnostic procedures and expenses for the health care system. It has been reported that ALS may cause elevated cTnT levels in the absence of cardiac impairment. To account for this, starting in November 2018 we systematically included cTnT levels in our work-up of patients with suspected or confirmed motor neuron disease at the Department of Neurodegenerative Diseases and Gerontopsychiatry at the University Hospital of Bonn. Our analysis of 85 consecutive cases comprised 59 patients with ALS (including 5 with genetic ALS mutations such as C9ORF72, TBK1 and SOD1)), 5 patients with Primary Lateral Sclerosis (PLS), 2 patients with benign fasciculation syndrome and 2 patients with inclusion body myositis (sIBM). As expected, 75% of the patients with ALS presented with cTnT levels above the 99th percentile cutoff at 14 ng/l. To better understand the basis of this finding we performed subgroup analyses and clinical correlations. The cTnT level in serum was negatively correlated with the scores in the ALS functional scale and positively correlated with CK-MB serum levels and CSF neurofilament (pNf-H) levels. Patients with PLS, benign fasciculation syndrome and pure bulbar ALS in contrast always showed low (<14 ng/l) or non-detectable cTnT serum levels.
We propose that cTnT elevation in MND are of non-cardiac origin and may serve as a marker of lower motoneuron or skeletal muscle involvement. cTnT levels may thus be helpful in defining restricted phenotypes of ALS such as PLS and bulbar ALS and may also have value as a prognostic marker. Further research is necessary to determine the biological origin of the cTnT elevation and to confirm its validity as a diagnostic and/or prognostic marker. Our finding also serves as a reminder to cautiously interpret cTnT elevations in patients with neuromuscular diseases.

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2079: **Neurofilaments as biomarkers to differentiate KD from ALS**.

Vittoria Lombardi PhD*(1,2), Bombaci Alessandro MD(1,3), Luca Zampedri(1), Ching-Hua Lu MD, PhD(2,4), Bilal Malik PhD(5), Henrik Zetterberg PhD(1,7,8,9), Amanda Heslegrave PhD(1,7), Carlo Rinaldi MD, PhD(6), Linda Greensmith PhD(1), Michael Hanna FRCP(1), Andrea Malaspina MD, PhD, FRCP(2) and Pietro Fratta MD, PhD(1).
1)Institute of Neurology, University College London Institute of Neurology, London, UK.
2)Blizard Institute, Queen Mary University of London, London, UK.
3)Department of Neuroscience "Rita Levi Montalcini", Molinette Hospital, University of Turin, Turin, Italy.
4)Department of Neurology, China Medical University Hospital, Taiwan.
5)Basildon Hospital, Basildon, UK.
6)Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.
7)UK Dementia Research Institute at UCL, London, UK.
8)Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.
9)Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden.

Background and aim: Kennedy disease (KD), also known as spinal-bulbar-muscular-atrophy (SBMA), is a progressive, adult-onset X-linked neuromuscular disease1. Although traditionally considered a motor neuron disorder (MND), recent advances have highlighted a primary myopathic component4,5. In order to identify novel biomarkers for KD, we evaluated levels of Neurofilament-Light-chain (NfL) and phosphorylated-Neurofilament-Heavy-chain (pNfH) as indicators of neuronal damage, and CK and creatinine levels as markers of muscle damage in KD patients and a mouse model of disease.
Materials and methods: We collected plasma and serum from 93 KD, 50 ALS and 50 healthy control cases, alongside with plasma from a mouse model of KD (AR100) and littermate controls. We measured NfL and pNfH plasma levels using Single-Molecule-Array (Simoa)3 and assessed CK and creatinine levels using standard laboratory testing. We analysed data using Kruskal-Wallis test and Cox regression analysis.
Results: Both NfL and NfH were elevated in ALS, as previously reported2, but, intriguingly, there was no change in KD patients. This finding was confirmed in the KD mice. ROC curves support the use of NfL and pNfH as a diagnostic biomarker to differentiate between these two disorders. Importantly, both CK and creatinine were significantly changed in KD vs controls, and creatinine changes correlated with disease severity.
Discussion and conclusions: This study finds, unexpectedly, that levels of neurofilaments are normal in KD, differently from MNDs2, whilst CK and creatinine are altered. These findings support the hypothesis of primary muscle damage in KD. In summary, neurofilaments could be used as biomarkers to differentiate KD from ALS and creatinine as a biomarker to evaluate disease progression in KD.

2083: **Stress-granules disassembly in health and ALS revealed by APEX proximity labelling proteomics .**

Hagai Marmor-Kollet (1), Naama Knafo (2), Natalia Rivkin (1), Aviad Siany (1), Nancy Kedersha (3), Tamar Geiger (2), Eran Hornstein (1)
(1) Weizmann Institute of Science, Rehovot, Israel.
(2) Tel Aviv University, Tel Aviv, Israel.
(3) Harvard Medical School, Boston, USA.

Stress granules are membraneless organelles, composed of RNA and RNA binding proteins, and control different aspects of RNA metabolism. Stress granules are associated with the pathology of ALS, as various ALS-causing mutations affect granules turnover and drive aberrant phase separation. We hypothesize that understanding the mechanisms for stress

granule will shed new light on the molecular pathology of ALS, including on the nucleation of insoluble aggregates that are present in patients brain and spinal cords. Here we use live cell imaging and APEX proximity labeling to characterize the stress granule proteome in an unbiased manner under cellular stress and ALS-associated C9orf72 proteotoxicity. We discovered and validated dozens of novel stress granule inhabitant proteins, some of which suggest intriguing new pathways into the molecular pathology of ALS. Direct proteomic measurements of stress granule disassembly dynamics should yield insight into the normal biology of phase-separated membraneless RNA organelles, and their aberrant persistence in disease.

2087: **Structural grey-and white matter pathology in ALS correlates with the D50 disease progression model as revealed in VBM and TBSS studies.**

Robert Steinbach*(1), Annika Voss(1), Nayana Gaur(1), Meerim Batyrbekova(1), Annekathrin Rödiger(1), Benjamin Ilse(1), Anne Gunkel(1), Beatrice Stubendorff(1), Thomas Mayer(3), Otto W. Witte(1,2), Tino Prell(1,2), Julian Grosskreutz(1,2).
1 Hans-Berger Department of Neurology, Jena University Hospital, Jena, Germany.
2 Center for Healthy Ageing, Jena University Hospital, Jena.
3 Department of Neuroradiology, Jena University Hospital, Jena, Germany.

Introduction: Mapping the progression of ALS-related neurodegeneration is an essential step to establish a neuroimaging biomarker for this devastating disease. However, correlations of structural changes in the brain and motorfunction-related symptom severity have been poor in former MRI studies. Therefore, parameters describing the time-course of ALS disease progression were ascertained via the D50 model from a large cohort of ALS patients to relate them to structural gray matter (GM) and white matter (WM) alterations.
Methods: T1-weighted (n=85) and DTI images (n=104) of ALS-patients as well as 63 healthy controls were analyzed. Voxel-based-morphometry (VBM) analyses were calculated via the CAT12 toolbox of SPM, Tract-Based Spatial Statistics (TBSS) were conducted with the FSL software package. The disease course was assessed with the D50 model, that e.g. allows an allocation to the current disease phase I or II (of I-IV) or a description of the disease-aggressivity (e.g. the D50-value = time until the ALSFRS-R drops to 24). Based on the D50 model parameters, the patient-cohort was divided into subgroups, as well as possible correlations examined in regression analyses.
Results: Using VBM, ALS-patients were characterized by density-reductions in GM and WM, pronounced in fronto-temporal brain regions. Patients of phase I and II showed different patterns of structural changes, indicating a leap of GM and WM pathology beyond the motor system along with the transition towards phase II. ALS patients with a higher disease aggressiveness (defined by a D50<30 months) showed supratentoriel white matter density decreases when compared with the slowly progressing patients, whilst there was no difference of GM density between these subgroups.
The widespread WM pathology in this ALS-cohort was also confirmed by TBSS results, that revealed decreased FA and increased MD/RD in multiple supra- and infratentorial tracts. Further regression analyses showed, that especially enhanced RD values were correlated with the disease phase and the calculated disease aggressiveness at the day of MRI acquisition.
Conclusions: In this cohort, the application of the D50 model was able to demonstrate a strong relation between symptom severity of ALS patients and in-vivo measures of cerebral structural integrity. We recommend to use the D50 model in studies trying to link clinical data with neuroimaging analyses or other assessments, especially for heterogenic cross-sectional cohorts.

2089: **Skeletal muscle MRI differentiates SBMA and ALS and correlates with disease severity**.

Uros Klickovic MD MRes (1,4), Luca Zampedri (1), Christopher DJ Sinclair PhD(3), Stephen J Wastling PhD(3), Karin Trimmel MD PhD(1), Robin JMW Howard MD(1), Andrea Malaspina MD PhD(2), Nikhil Sharma MD PhD(1), Katie CL Sidle MD(1), Ahmed Emira(3), Sachit Shah MD(3), Prof Tarek A Yousry MD(3), Prof Michael G Hanna MD(1), Prof Linda Greensmith PhD(1), Jasper M Morrow MD PhD(1), John S Thornton PhD(3),* and Pietro Fratta MD PhD(1)*.
1.UCL Queen Square Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK.
2.Blizard Institute, Queen Mary University of London, London, E12AT, UK.
3.Neuroradiological Academic Unit, UCL Queen Square Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK.
4.Department of Radiology, University Hospital Tulln, Karl Landsteiner University of Health Sciences, Tulln, Austria.

We investigated muscle MRI as a tool for the differential diagnosis and as a disease progression biomarker for two major forms of motor neuron disorders, spinal bulbar muscular atrophy (SBMA - also known as Kennedy's disease) and amyotrophic lateral sclerosis (ALS).
We performed quantitative 3-point Dixon, semi-quantitative T1-weighted and STIR imaging to bulbar and bilateral thigh and calf muscles in parallel to clinical and functional assessments in ALS (n=21), SBMA (n=21) patients, and healthy controls (n=16). We analyzed the presence of fat infiltration and edema, as well as patterns of muscle involvement. We finally correlated clinical parameters of disease severity in ALS and SBMA with the quantitative MRI measurements.
Our work revealed significant fat infiltration in bulbar and limb muscles in SBMA compared to controls, identifying a specific, previously unkown, pattern of muscle involvement. Semi-quantitative STIR imaging detected marked hyperintensities in ALS lower limb muscles, distinguishing ALS from SBMA and controls. Lastly, MRI measurements correlated significantly with clinical scales of disease severity in both ALS and SBMA.
Our findings show that muscle MRI differentiates SBMA and ALS and correlates with disease severity, supporting its use as a diagnostic tool and biomarker for disease progression. This work underlines the clinical utility of muscle MRI in motor neuron disorders and contributes to establish novel objective outcome measures, which are crucial for effective clinical trials.

2091 **The distribution of polyunsaturated fatty acids in lipids from blood cell pellets: a potential biomarker for amyotrophic lateral sclerosis.**

Laura Robelin (1, 2), Alexandre Henriques (1, 2), François Salachas (3), Hélène Blasco (4, 5, 6), Gabrielle Rudolf (7, 8), Martine Bergaentzle (9), Marie-Céline Fleury (1, 2, 7), Christian Andres (4, 5), Philippe Corcia (4, 10), Eric Marchioni (9), Jean-Philippe Loeffler (1, 2), Pierre-François Pradat (3, 11) Jose-Luis Gonzalez De Aguilar * (1, 2).
1 Université de Strasbourg, UMR_S1118, Strasbourg, France.
2 INSERM, U1118, Mécanismes Centraux et Périphériques de la Neurodégénerescence, Strasbourg, France.
3 Assistance Publique - Hôpitaux de Paris, Hôpital de la Pitié-Salpêtrière, Fédération des Maladies du Système Nerveux, Centre Référent Maladie Rare SLA, Paris, France
4 INSERM, U930, Neurogénétique et Neurométabolomique, Tours, France.
5 Université François-Rabelais, UMR_S930, Tours, France.
6 CHRU Tours, Laboratoire de Biochimie et de Biologie Moléculaire, Tours, France.
7 Hôpitaux Universitaires de Strasbourg, Service de Neurologie, Strasbourg, France.
8 Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM U1258, CNRS UMR 7104, Université de Strasbourg, Illkirch, France.
9 Chimie Analytique des Molécules BioActives, Institut Pluridisciplinaire Hubert Curien, UMR 7178 CNRS/Université de Strasbourg, Illkirch, France.
10 Centre Hospitalier Universitaire Bretonneau, Centre SLA, Tours, France.

11 Sorbonne Universités, UPMC Université Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France.

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease associated with yet unexplained systemic metabolic disarrangements. Here we determined the distribution of polyunsaturated fatty acids (PUFAs) in total lipids extracted from blood cell pellets to establish potential biomarkers. Levels of docosapentaenoic acid (DPA, 22:5n-3) and arachidonic acid (AA, 20:4n-6), as well as relative indices of 3 and 6 PUFA status, decreased significantly in ALS patients compared to healthy control subjects. In particular, reduced DPA levels best characterized ALS individuals. We obtained similar results by comparing between an independent set of ALS men and patients with spinal and bulbar muscular atrophy (SBMA), which is another motor neuron disease also associated with metabolic disturbances. In this case, a reduced ratio of AA to linoleic acid (LA, 18:2n-6) best distinguished between ALS and SBMA patients. We also found that levels of dihomo-gamma-linolenic acid (DGLA, 20:3n-6) were an independent prognostic factor for survival. These findings strongly suggest that the analysis of the PUFA composition of blood cell lipids can be a relevant biomarker for ALS in clinical practice and therapeutic trials.

2095 **Dysregulated biosynthesis of long-chain fatty acid in ALS outlines prognostic biomarkers**.

Francisco Gil-Bea*, Gorka Fernández-Eulate, José Ignacio Ruiz-Sanz, Javier Riancho, Ahmad Al Khleifat, Nick J Ashton, Abdul Hye, Monica Zufiría, Gorka Gereñu, Roberto Fernández-Torrón, Juan José Poza-Aldea, Jon Ondaro, Marta Urtizberea, Juan Bautista Espinal, Gonzalo González-Chinchón, Montse Ruiz, Miren Zulaica, Aurora Pujol, Maria Begoña Ruiz-Larrea, Ammar Al-Chalabi, Adolfo López de Munain.
ALS multidisciplinary clinic (UMELA), Hospital Universitario Donostia
Biodonostia Research Institute, San Sebastián.

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal motor neuron disease. Some clinical and experimental evidences suggest abnormal lipid profiles in ALS that may stem from altered synthesis and oxidation of fats. In this study we perform a comprehensive profiling of the serum lipidome to increase understanding of metabolic abnormalities in amyotrophic lateral sclerosis (ALS) and seek potential biomarkers. A total of 71 people (39 with ALS and 32 healthy controls) were enrolled in a cross-sectional study. 23 ALS patients were followed up for an interval of 2.5 years from symptom onset. Fasting serum lipid profile was quantified by ultrahigh-performance liquid chromatography-mass spectrometry and gas chromatography-flame ionization detector. Lipidomic data was analyzed by univariate and multivariate methods. Age- and sex-adjusted Cox proportional hazards regression was used to estimate hazard ratios of serum lipids on mortality and outcome events of ALS clinical staging: percutaneous endoscopic gastrostomy (PEG) and non-invasive mechanical ventilation (NIV). Results were compared with Neurofilament light chain ELISA analysis. 28 of 416 lipids, mostly triacylglycerides and esphingolipids, were altered in ALS. The analysis of fatty acid (FA) content revealed a decreased delta-9 desaturation activity and an increased ELOVL6-dependent elongation of C14 and C16 FAs, which correlated with higher levels of omega-9 monounsaturated FAs in ALS. These specific changes were reproduced in cell lines upon genetic inhibition of two ALS genes: TARDBP and FUS. Remarkably, reduced levels of serum C14:0 and C16:1n-7 FAs were associated with increased hazard ratio of mortality (HR=15.17, p=0.001), PEG (HR=36.91, p=0.003) and NIV (HR=5.03, p=0.009) in ALS patients. These lipids showed a better predictive value than NfL (mortality: HR=4.05, p=0.085; PEG: HR=8.64, p=0.045; NIV: HR=1.79, p=0.057). In conclusion, alterations in the metabolism of long-chain FAs that

impinge on the biosynthesis of omega-9 FAs may provide a compelling explanation for the lipid abnormalities of ALS, and outline biomarkers to monitor ALS progression.

2099: **Cerebellar pathology in ALS: a prospective neuroimaging study**.

Stacey Li Hi Shing, Rangariroyashe Hannah Chipika, Eoin Finegan, Orla Hardiman, Peter Bede.
Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin.

Background: The neuroimaging signature of ALS is primarily associated with motor cortex, pyramidal tract and corpus callosum alterations. Consistent with the neuropsychological profile of ALS, a significant proportion of patients also exhibit widespread extra-motor pathology in frontotemporal and subcortical grey matter regions. Cerebellar involvement is difficult to ascertain clinically in ALS due to concomitant pyramidal and lower motor neuron degeneration. Accordingly, the objective of this study is the characterisation of cerebellar pathology using multiparametric neuroimaging.
Methods: Seventy-five patients and seventy-fine healthy controls were enrolled into a prospective neuroimaging study. Whole-brain and region-of-interest (ROI) grey and white matter analyses were performed assessing cerebellar grey and white matter indices; cortical volumes, fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD). All comparisons were corrected for age and in addition to group-level analyses the distribution profile of imaging metrics was also evaluated in the patient cohort.
Results: Whole-brain analyses revealed bilateral cerebellar FA reductions and increased RD at $p < 0.01$ FWE. While grey matter morphometry did not capture statistically significant alterations at a 'whole-brain' level, region-of-interest analyses revealed cerebellar atrophy in the vermis, lobule V and crus II. The histogram of cerebellar grey matter volumes suggests a bimodal distribution, indicating that a sub-cohort of ALS patients exist with marked cerebellar atrophy. ROI white matter analyses confirmed cerebellar FA reductions ($p = 0.012162$) and increased RD ($p = 0.017268$). The distribution profile of cerebellar FA values is also suggestive of a subgroup of patients with particularly low FA.
Conclusions: ALS is associated with considerable cerebellar involvement, which is dominated by white matter pathology and best detected by region-of-interest analyses. Cerebellar degeneration in ALS is challenging to detect clinically due to co-existing motor neuron degeneration and is relatively under recognised. Our study confirms extensive cerebellar pathology, which may contribute to gait impairment, falls, impaired dexterity, dysarthria increasing the heterogeneity of clinical presentations observed in ALS.

CLINICAL:

1903: **Implications of spirometric reference values for amyotrophic lateral sclerosis.**

Ruben P.A. van Eijk MD (1,2), Jaap N.E. Bakers MSc (3), Michael A. van Es MD (1), Marinus J.C. Eijkemans PhD (2), Leonard H. van den Berg MD (1).
(1) Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, the Netherlands.
(2) Biostatistics & Research Support, Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, the Netherlands.
(3) Department of Rehabilitation, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, the Netherlands.

Background: Spirometry is commonly used as screening tool for respiratory insufficiency in neuromuscular diseases. We aim to assess the effect of spirometric reference values on prognostication, medical decision-making and trial eligibility in amyotrophic lateral sclerosis (ALS).
Methods: We selected 4,651 patients with 32,022 FVC measurements from the PRO-ACT dataset. The FVC estimates were standardized according to five reference standards: Knudson '76, Knudson '83, ECSC, NHANES III and GLI-2012. (Generalized) linear mixed-effects and Cox proportional hazard models were used to evaluate longitudinal patterns and time-to-event outcomes.
Results: The mean population %predicted FVC varied between 78.5% (95% CI 78.0-79.1) and 88.5% (95% CI 87.9-89.1). The unstandardized litres provided the worst fit on the survival data (AIC 20573, c-index 0.760), whereas the GLI provided the best fit (AIC 20374, c-index 0.780, $p < 0.001$). The mean population rate of decline in %predicted FVC could vary as much as 11.4% between reference standards. The median time-to-50% predicted FVC differed by 2.9 months between recent (14.5 months, 95% CI 14.4 – 16.1) and early reference standards (17.4 months, 95% CI 16.1 – 18.2).
Conclusion: Spirometric reference values affect the utility of spirometry in ALS and, potentially, other neurological diseases. Standardization may optimize clinical decision-making, improve prognostication, enhance between-centre comparison and unify patient selection for clinical trials.

1913: **A ferroptosis-based panel of prognostic biomarkers for Amyotrophic Lateral Sclerosis**.

AS Rolland (1)*, D Devos (1,2), C Moreau (2), M Kyheng (3), G Garçon (4), H Blasco (5), P Gelé (6), T Lenglet (7), Veyrat-Durebex(5), P Corcia (8), M Dutheil (2), P Bede (9,10), A Jeromin (11), P Oeckl (12), M Otto (12), V Meninger (9), V Danel (1), C Devedjian (2), JA Duce (14,15), PF Pradat (16).
(1) Département de Pharmacologie Médicale, Université de Lille, INSERM UMRS_1171, Centre Hospitalier Universitaire, Centre COEN LICEND, Lille, France
(2) Département de Neurologie, Centre ALS, Université de Lille, INSERM UMRS_1171, Centre Hospitalier Universitaire, Centre COEN LICEND, Lille, France
(3) Département de Biostatistiques, Université de Lille, Centre Hospitalier Universitaire, Lille, France
(4) Université de Lille, CHU Lille, Institut Pasteur de Lille, EA 4483 IMPECS-IMPact de l'Environnement Chimique sur la Santé humaine, France
(5) Université François-Rabelais, Inserm U930, Laboratoire de Biochimie, CHRU de Tours, France
(6) CRB/CIC1403, Université de LILLE
(7) APHP, Department of Neurophysiology, Pitié-Salpêtrière Hospital, Paris, France
(8) Centre Constitutif SLA, Tours-Fédération des centres SLA Tours-Limoges, LITORALS
(9) Ramsay, Hôpital des Peupliers, Paris, France
(10) Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin, Ireland
(11) Quanterix, Lexington, Massachusetts, USA

(12) Department of Neurology, Ulm University Hospital, Oberer Eselsberg 45, 89081 Ulm, Ulm, Germany
(13) APHP, Department of Neurology, Paris ALS Center, Pitié Salpêtrière Hospital, France
(14) Alzheimer's Research UK Cambridge Drug Discovery Institute, University of Cambridge, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0AH, UK.
(15) Oxidation Biology Unit, The Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Parade, Parkville, 3052, Victoria, Australia.
(16) Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France; APHP, Département de Neurologie, Hôpital Pitié-Salpêtrière, Centre référent SLA, Paris, France.

Accurate patient stratification into prognostic categories and targeting Amyotrophic Lateral Sclerosis (ALS)-associated pathways may pave the way for promising trials. We evaluated blood-based prognostic indicators using an array of pathological markers. Plasma samples were collected as part of a large, phase III clinical trial (Mitotarget/TRO19622) at months 1, 6, 12 and 18. The ALSFRS-r score was used as a proxy of disease progression to assess the predictive value of candidate biological indicators. First, established clinical predictors were evaluated in all 512 patients. Subsequently, pathologic markers, such as proxies of neuronal integrity (Neurofilament light chain and phosphorylated heavy chain), DNA oxidation (8-oxo-2'-desoxyguanosine), lipid peroxidation (4-hydroxy-2-nonenal, isoprostane), inflammation (interleukin-6) and iron status (ferritin, hepcidin, transferrin) were assessed in a subset of 109 patients that represented the whole cohort.
Markers of neuronal integrity, DNA and lipid oxidation, as well as iron status at baseline are accurate predictors of disability at 18-month follow-up. The composite scores of these markers in association with established clinical predictors enable the accurate forecasting of functional decline. The identified four biomarkers are all closely associated with 'ferroptosis', a recently discovered form of programmed cell death with promising therapeutic targets. The predictive potential of these pathophysiology-based indicators may offer superior patient stratification for future trials, individualised patient care and resource allocation.

1942: **Confirmation that the C9orf72 expansion is associated with accelerated respiratory function decline in Amyotrophic Lateral Sclerosis.**

James Rooney(1), Deirdre Murray(1), Anna Campion(1), Hannah Moloney(1), Rachel Tattersall(1), Mark Doherty(1), Michaela Hammond(1), Mark Heverin(1), Russell McLaughlin(1), Orla Hardiman(1, 2).
1. Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Dublin 2, Ireland
2. Beaumont Hospital, Beaumont Road, Dublin 9, Ireland.

Introduction: The C9orf72 hexanucleotide repeat expansion is causal in ALS and has a negative effect on prognosis. Recently the C9orf72 repeat expansion was associated with an accelerated deterioration of respiratory function and survival in a cohort of 372 Portuguese patients [1].
Methods: Cases presenting to the Irish ALS MDT with both longitudinal Sniff Nasal Inspiratory Pressure (SNIP) and C9orf72 testing were including in the present study. Clinical variables and survival characteristics of these patients were collected. Joint longitudinal and time to event models were constructed to explore the longitudinal characteristics of the cohort by C9orf72 status.
Results: Six hundred and thirty cases were included and 58 (9.2%) carried the C9orf72 repeat expansion. In a Cox survival model age of onset, diagnostic delay, bulbar onset and C9orf72 status were all prognostic. Plots of the longitudinal trend after joint modelling revealed that those carrying the expansion had a worse respiratory function throughout the course of their disease than those without. Furthermore, modelling by site of onset and sex sub-groups revealed that this difference was maximal in male spinal onset cases.

Discussion: Our results confirm findings in the Portuguese cohort that the C9orf72 repeat expansion is associated with accelerated respiratory function decline. Furthermore, our finding that male spinal onset patients with the C9orf72 repeat expansion suffer faster respiratory decline than others is congruent with previous findings form 5 European cohorts that male spinal onset C9orf72 expansion carrying patients suffer a worse prognosis than others[2].

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1960: **AriSLA, the Italian Foundation for ALS research: mission, vision, and outcomes of ten-year investment.**

Maddalena Ravasi*(1), Stefania Guareschi*(1), Luca Maria Munari (1), Anna Ambrosini (1).
1) AriSLA, Fondazione Italiana di ricerca per la SLA - Sclerosi Laterale Amiotrofica, Milan, Italy.
*These authors equally contributed.

AriSLA, the Italian Foundation for ALS research, was founded by AISLA Onlus (the main ALS Italian patient organisation) with Cariplo, Telethon and Vialli e Mauro foundations. Its mission is to support Italian excellent scientific research towards the cure of ALS. To reach this goal, AriSLA is working together with the Italian scientific community, playing the role of catalyst and driving force by providing economical, scientific and technical support.
Since its start in 2009, AriSLA adopted a rigorous peer review system to select the best research projects mainly through a bottom-up approach. Overall, AriSLA issued 11 calls for proposals, with an investment of more than 11 million euros. It funded 41 full grants, projects with a solid background and consistent preliminary data, and 31 pilot grants, 1-year projects with highly innovative hypotheses and few or not available preliminary data. These projects targeted basic research (52% of funds), preclinical and translational (27%), clinical (15%) and technological (6%) areas.
AriSLA performs a constant scientific and administrative monitoring of its funded projects. Moreover, it organises research training and promotes dissemination of scientific and lay information on ALS within the scientific community and the general public, respectively.
A recent research portfolio and benchmark analysis highlighted relevant outcomes of this regular investment and a number of "success stories", which will be discussed in the poster.
Bibliometric analysis performed using the NIH Relative Citation Ratio (RCR) index (https://icite.od.nih.gov/stats) showed that the 63% of the publications derived from AriSLA funded projects falls in the top 50% of NIH RCR percentiles, with 23% in the top 20% (27 original papers and 13 reviews).
Regarding the pilot grant program, it attracted several investigators new to the ALS field (20 out of 31) and supported many investigators younger than 40 years of age (39%). Moreover, 11% of the pilot projects proved successful in subsequent AriSLA full grants.
AriSLA holds annual symposia to update its stakeholders (patients and investigators) on the latest research updates derived from its funded projects; since 2015, these meetings are co-organized with AISLA.
Conclusion: AriSLA independent peer review process proved effective to select excellent research, with high impact on ALS field, as demonstrated by the high RCR scores and the consolidated networking activity.

1963: **An FTLD case with a TUBA4A mutation and TDP-43 pathology**.

Evelien Van Schoor (1,2)*, Mathieu Vandenbulcke (3), Rik Vandenberghe (4,5), Julie van der Zee

(6,7), Christine Van Broeckhoven (6,7), Philip Van Damme (2,5), Ludo Van Den Bosch (2), Dietmar R. Thal (1,8).
(1) Laboratory for Neuropathology, Department of Neurosciences, KU Leuven (University of Leuven), Leuven Brain Institute (LBI), Leuven, Belgium.
(2) Laboratory for Neurobiology, Department of Neurosciences, KU Leuven (University of Leuven) and Center for Brain & Disease Research, VIB, Leuven Brain Institute (LBI), Leuven, Belgium.
(3) Section of Old Age Psychiatry, Department of Psychiatry, University Hospitals Leuven, Leuven, Belgium.
(4) Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven (University of Leuven), Leuven, Belgium
(5) Department of Neurology, University Hospitals Leuven, Leuven, Belgium.
(6) Neurodegenerative Brain Diseases Group, Center for Molecular Neurology, VIB, Antwerp, Belgium.
(7) Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.
(8) Department of Pathology, University Hospitals Leuven, Leuven, Belgium.

In the past, disease-associated variants of the TUBA4A gene were identified in patients with familial ALS, some of whom had signs of cognitive impairment. The TUBA4A gene encodes for the alpha tubulin 4A protein, which polymerizes with _-tubulin to form the structural subunits of microtubules. We present the case report of a 60-year old man diagnosed at the age of 51 with the semantic variant of frontotemporal lobar degeneration (FTLD), which was confirmed by MRI and PET. The patient did not show signs of motor neuron disease. There was a family history of Parkinson's disease on the father's side. Genotyping revealed a novel frameshift mutation c.187del (p.Arg64Glyfs*90) in exon 2 of TUBA4A, which was absent from control individuals and public databases.
Theoretically, the frameshift mutation in this FTLD case does not lead to nonsense-mediated mRNA decay, but to the translation of a truncated protein. However, Western blot using an N-terminal TUBA4A antibody did not reveal a TUBA4A fragment. When investigating full-length TUBA4A, we noticed an increase in TUBA4A protein expression compared to controls and sporadic FTLD cases. Immunohistochemistry (IHC) showed abundant TAR DNA-binding protein 43 kDa (TDP-43) pathology primarily in the frontal and temporal cortex and the dentate gyrus of the hippocampus, consistent with FTLD. However, the observed pathology did not classify as one of the proposed FTLD-TDP types (Lee et al., 2017), as pTDP-43 IHC showed long thin but also short thick threads and cytoplasmic inclusions. Additionally, TDP-43 lesions were spread over all cortical layers with more prominent pathology in layers II and V. Furthermore, TUBA4A IHC showed an intense and divergent staining. Thick neurites positive for TUBA4A not co-localizing with pTDP-43 threads were a prominent feature, whereas this type of thickened TUBA4A-positive neurites were not observed in control cases.
To our knowledge, this is the first TUBA4A mutation identified in a patient with pure FTLD without any motor neuron phenotype. Furthermore, histopathological and biochemical analysis of the frontal and temporal cortex shows TUBA4A-associated neurodegeneration that differs from that of other FTLD cases. This implies that TUBA4A might be another genetic cause for both ALS and FTLD.

1967: **Exploration of Stability of Apathy Subtypes in Motor Neurone Disease.**

Ratko Radakovic* (1, 3, 4), Debbie Gray* (2, 3, 4), Kaitlin Dudley (1), Eneida Mioshi (1), David Dick (5), Helen Copsey (5), Giulia Melchiorre (3, 4), Harry Gordon (3, 4), Judith Newton (3, 4), Shuna Colville (3, 4), Suvankar Pal (3, 4), Siddharthan Chandran (3, 4), Zachary Simmons (6), Sharon Abrahams (2, 3, 4).
1 Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK.
2 Department of Psychology, University of Edinburgh, Edinburgh, UK.
3 Euan MacDonald Centre for Motor Neurone Disease Research, Royal Infirmary of Edinburgh, Edinburgh, UK.
4 Anne Rowling Regenerative Neurology Clinic, Royal Infirmary of Edinburgh, Edinburgh, UK.

5 Norfolk and Norwich University Hospital, Norwich, UK.
6 Department of Neurology, Pennsylvania State University, Hershey, Pennsylvania, USA.

Background: As the most prevalent non-motor symptom in motor neurone disease (MND), apathy is multidimensional construct quantifiable by the Dimensional Apathy Scale (DAS). Initiation apathy (a lack of motivation for self-generation of thoughts and/or actions) is a characteristic impairment in MND. However, the stability and fluctuation of apathy subtypes during the disease have not yet been examined.
Objectives: The aim was to determine the stability, prevalence and degree of apathy subtypes in MND over two different time-points.
Methods: 37 MND patients and 37 of their caregivers were recruited from Scotland and England, as a part of the Clinical Impact of Apathy in MND (CIAMND) longitudinal study. Participants took part in interviews at baseline and follow-up (3 months later) where they completed measures of apathy profiling (DAS), as well as mood (Patient Health Questionnaire-9, Generalised Anxiety Disorder Questionnaire-7), emotional lability (Emotional Lability Questionnaire), cognitive functioning and behavioural change (Edinburgh Cognitive and Behavioural ALS Screen) and functional disability (ALS Functional Rating Scale-Revised). Comparative analyses was performed on scores and frequency of impairment (based on clinical cut-offs) over two time-points.
Results: 46% of patients displayed increased apathy for at least one subtype at either time-point. Over these time-points, 32% patients had stable (apathy present at baseline AND follow-up), 11% had increased and 11% had decreased Initiation apathy, which was significantly different from Executive (stable=8%; increased=8%; decreased=5%) and Emotional (stable=8%; increased=8%; decreased=5%) apathy ($p<.05$). Depression and anxiety measures showed different stability profiles. Further, analyses of scores revealed no significant change in Initiation, Executive or Emotional apathy scores from baseline to follow-up, indicative of no fluctuation. Similar findings were observed in relation to mood, anxiety, emotional lability, cognitive functioning and behaviour.
Conclusions: Apathy subtypes were observed to be 46% prevalent and did not fluctuate over two time-points. Initiation apathy as the most common demonstrated marginally more significant stability in terms of clinical cut-offs. This has implications for non-pharmacological interventions and further research should explore fluctuations and interactions of apathy subtypes longitudinally in relation to quality of life, wellbeing and burden.

1969: **Evaluation of screening tests for dysphagia in motor neuron disease.**

Elisabet Romero (1,2), Maria-Antonia Barceló* (2,3,4), Raúl Dominguez (2,5), Núria Pérez (6), Núria Virgili(1,2), Monica Povedano(2,5), Marc Saez (3,4).
1. Department of Endocrinology and Clinical Nutrition, Bellvitge University Hospital (HUB), L'Hospitalet de Llobregat, Spain.
2. Functional Unit of Motor Neuron Disease (UFMN), Bellvitge University Hospital (HUB) – Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain.
3. Research Group on Statistics, Econometrics and Health (GRECS), Universitat de Girona, Girona, Spain.
4. CIBER in Epidemiology and Public Health (CIBERESP), Spain.
5. Department of Neurology, Bellvitge University Hospital (HUB), L'Hospitalet de Llobregat, Spain.
6. Department of Radiology, Bellvitge University Hospital (HUB), L'Hospitalet de Llobregat, Spain.

Rationale Deglutition disorders are common in patients with motor neuron disease (MND). Oropharyngeal dysphagia can occur in 80% of patients, and onset could be early, at the diagnosis of the disease (bulbar forms), or in later stages of evolution (in spinal forms). Dysphagia contributes negatively in the evolution of motor neuron disease due to its affectation on the patient's nutritional status and respiratory capacity. An early diagnosis of

oropharyngeal dysphagia would allow a better nutritional approach, which in turn would improve the quality of life of the patient. Currently, there are no specific clinical tests for the diagnosis of dysphagia in MND. In this study, we aimed to asses both, the specificity and the sensitivity of the dysphagia screening tests available for other diseases (EAT-10), as well as to compare the specific swallow assessment scale for ALS (ALS-SS) results with videofluoroscopy (gold standard technique for the diagnosis of dysphagia). Methods From 154 patients visited in the Functional Unit of MND of the Hospital Universitari de Bellvitge, between September 2017 and July 2018, with different status progression, and who met inclusion criteria, 46 agreed to participate in our study. 52.17% were women; bulbar affection was in 78.3% patients which 30.6% with bulbar onset and 69.4% in later stages of evolution. The dysphagia was assessed in all patients with videofluoroscopy and dysphagia screening tests: EAT10 and subscale ALS-SS. Participants were also asked to fill out a quality of life questionnaire (SwalQoL), from which a selection was made of the 7 items that were considered of greatest interest for the assessment of dysphagia. Results Compared with videofluoroscopy, ALS-SS subscale showed a better specificity (87.5%) and sensitivity (72.9%) than EAT10, which specificity and sensitivity were 70.3% and 75.0% respectively. SwalQoL 7 items selection showed a specificity of 100% and a sensitivity of 50%. Conclusions ALS-SS gives similar results to those of videofluoroscopy.

1971: **Behavioural disturbances in motor neuron disease: longitudinal validation of the ALS-FTD-Questionnaire.**

Rosanne Govaarts* (1), Emma Beeldman (1), Mariëlle de Vreede (1), Ruben van Eijk (2), Hepke Grupstra (3), Ben Schmand (4), Gert Geurtsen (4), Rob de Haan (5), Marianne de Visser (1), Joost Raaphorst (1).

1 Department of Neurology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
2 Department of Neurology, Rudolf Magnus Institute, UMC Utrecht, Utrecht, The Netherlands.
3 Department of Rehabilitation, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
4 Department of Medical Psychology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
5 Clinical Research Unit, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

Behavioural disturbances are present in up to 50% of patients with motor neuron disease (MND). Despite several longitudinal studies, the course of behavioural disturbances in MND is unclear; none of the disease-specific behavioural questionnaires have been validated longitudinally. The ALS – frontotemporal dementia- questionnaire (ALS-FTD-Q) has been validated in a cross- sectional design in ALS patients, including construct validity.
The aims of this study included: 1. to assess the construct validity of the ALS-FTD-Q in a longitudinal study design; and 2. to examine the responsivity of the ALS-FTD-Q.
To assess construct validity ALS-FTD-Q scores were correlated with measures of behaviour (with a correlation expected to be high), executive functioning (moderate), anxiety (low), depression (low), vital capacity (low), and motor impairment (low). To examine responsivity, first we investigated if the ALS-FTD-Q detects changes in behavioural disturbances over time using linear mixed-effect models. Second, we divided 3 subgroups based on the reliable change index of the ALS-FTD-Q score on the first and last visit; increased score, decreased score, and stable score.
We included 78 MND patients (58 ALS; 10 progressive muscular atrophy (PMA), 10 ALS-FTD). Median study duration was seven months (3-37) and the median number of visits was three (2-10). Construct validity was assessed at baseline (n=78) and after 5-7 months (n=61). At both assessments, correlation patterns between ALS-FTD-Q with measures of behaviour, executive functioning, anxiety, depression, vital capacity and motor impairment largely supported the construct validity of the ALS-FTD-Q. Regarding responsivity, the ALS-

FTD-Q showed no difference in slope of behavioural disturbances between ALS, PMA and ALS-FTD patients. Visual inspection of 3 subgroups was based on the presence and direction of change of behavioural disturbances, between baseline and last visit and showed groups with increased scores (15%), decreased scores (14%) and stable scores (71%). A high intra- and inter-individual variability between visits was observed.
In conclusion, the ALS-FTD-Q shows responsivity and a stable and acceptable construct validity over 6 months. Behavioural disturbances as measured with the ALS-FTD-Q seem not progressive in the majority of MND patients. Our results further suggest a considerable intra-and inter-individual variability in the course of behavioural changes in MND over time.

1975: **A patient with progressive spastic paraparesis, left temporopolar atrophy, neurocognitive deficits and fatigue: A case report**.

Maria Janina Wendebourg* (1), Nicole Naumann (1), Johannes Lorscheider (1), Marc Sollberger (1,2), Kathi Schweikert (1).
1. Department of Neurology, University Hospital Basel, Basel, Switzerland
2. Memory Clinic, University Center for Medicine of Aging, Felix Platter-Hospital, Basel, Switzerland.

Background: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are considered entities of a neurodegenerative spectrum of diseases that overlap and may show a broad range of symptoms (Strong et al., 2017). Case presentation: We report a 56-year old Swiss-German patient without family history of ALS or FTD presenting with weakness and muscle cramps of the left lower extremity (LE), fatigue and memory disturbances that had developed within 18 months. Clinical examination showed a spastic paresis of the left LE with brisk deep tendon reflexes without sensory impairment. Cerebral MRI showed white matter lesions without contrast enhancement fulfilling 2/4 Barkhof criteria. Spinal MRI revealed no abnormalities. Visually evoked potentials showed an increased P100-latency of the left visual afference. Motor evoked potentials showed an increased central motor latency to the left LE. Somatosensory evoked potentials and nerve conduction studies were normal. Electromyography showed signs of chronic denervation in the left tibialis anterior muscle. CSF analysis showed one oligoclonal band. Neurofilament light chain serum levels were elevated (237.7pg/ml(G; p.Asp183Gly). Conclusions: This case presents an interesting symptom evolution in a patient with ALS-FTD spectrum disorder. Imaging shows progressive atrophy of the left temporal pole. Clinically, disabling progression of motor symptoms, but only a slight decline of cognitive functions has been documented. Further planned analyses include family testing for co-segregation of the detected mutation and genetic testing for hereditary spastic paraparesis.

1977: **Occurrence of refeeding syndrome in motor neuron disease patients who undergo gastrostomy tube placement – a prospective clinical study**.

Katarina Vogelnik* (1), Lea Leonardis (1), Bla_ Koritnik (1), Leja Dolenc Gro_elj (1), Bernard Megli_ (2), Gregor Brecl Jakob (2), Stanka Risti_ Kova_i_ (1), Polona Klinar (1), Janez Zidar (1).
1 Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia.
2 Department of Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia.

Objective: To improve 30-day survival of motor neuron disease (MND) patients following gastrostomy tube (GT) placement and to evaluate the incidence of refeeding syndrome in these patients we monitored all MND patients undergoing GT insertion for signs of refeeding syndrome (RFS), regardless of the patient's individual risk of RFS.
Background: RFS is a condition characterized by potentially fatal shifts in fluids and electrolytes that may happen when artificial feeding is started in malnourished patients.

MND patients are prone to rapid weight loss and malnourishment due to multiple causes including dysphagia and increased metabolism. Although MND patients who undergo GT insertion often meet criteria for identifying patients at high risk of refeeding problems, the incidence of RFS in MND patients and its impact on patients' survival after GT insertion is poorly studied. With PubMed database search, we were able to find only one study in which MND patients who underwent GT placement were monitored for signs of RFS. In this study, no signs of refeeding syndrome were found in any of the studied patients.
Methods: Consecutive MND patients referred to our clinic for GT placement were included and no exclusion criteria were used. After GT placement nutrition support was commenced at 10kcals/kg body weight and was slowly increased over 4-7 days. All patients were instructed to take thiamine, vitamin B-complex preparation and a multivitamin-mineral preparation for 10 days, starting at least 1 day before the feeding was started. Serum magnesium, potassium, phosphorus and calcium levels were monitored daily for at least 4 days after GT placement.
Results: Over a period of 22 months, 27 patients were included. There were no deaths within the first 30-days following GT placement. At the time of GT placement patients' mean ALS-FRS score was 24.9 (SD 8.0), mean BMI was 23.0 (SD 4.0) and mean PaC02 was 5.5 (SD 0.62). Eight patients were using non-invasive ventilation and 1 patient was invasively ventilated. After the feeding was started, electrolyte disturbances were found in 19 patients (70.4%). In 10 patients (35.7%), electrolyte levels were significantly decreased and electrolyte supplementation was needed.
Conclusions: We believe that RFS after GT placement in MND patients is a common but often overlooked condition. By raising awareness of the high incidence of RFS in this group of patients, we might further improve their 30-day post-procedure survival.

1985: **The Role of Relationship Quality and Social Support on Motor Neuron Disease (MND) Caregivers Psychological Wellbeing.**

Síle Carney* (1) (2), Caroline Wheeler (1) (2), Mark Heverin (1), Miriam Galvin (1), Niall Pender (1) (2), Orla Hardiman (1) (3).
(1) Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.
(2) Department of Psychology, Beaumont Hospital, Dublin, Ireland.
(3) Department of Neurology, Beaumont Hospital, Dublin, Ireland.

Background: The experience of caregiving in the context of motor neuron disease (MND) is challenging, and there are limited data on the impact of quality and role of relationships, and social support on the caregivers' psychological wellbeing.
Methods: Semi-structured interviews at home visits collected data using the Positive Negative Relationship Quality (PNRQ) Scale, the Medical Outcomes Study-Social Support Survey and standardised measures of burden (Zarit Burden Interview), depression (PHQ-9), anxiety (GAD-7) and quality of life (QoL) (QOLLTI-v2). Four independent multiple regressions were performed to explore whether relationship quality and social support predict psychological distress in 83 informal caregivers.
Results: The relationship to the patient is a significant predictor of increased burden and anxiety in caregivers. Reduced social support is a significant predictor of caregiver burden. Negative relationship quality significantly predicts increased burden, anxiety and reduced QoL, whilst positive relationship quality significantly predicts increased QoL.
Conclusions: The quality and outcomes of caregiving are determined by the type of relationship between the patient and the caregiver. Recognition of this dynamic will support development of more effective interventions for caregivers. Additional insight can be drawn from the nature of the premorbid relationship and the change in relationship over the course of the illness.

1986: **Mental Health Support Plans For People Affected by MND.**
Andrew Bethell: NHS Highland.

The author would like to present the initial stages of a service development project intended to provide a support plan for carers of people affected by MND.
Approximately 10% of people with MND may suffer from a mental health issue such as apathy (Radakovic, 2018) depression and anxiety during the course of their disease. However over 30% of carers experience significant mental health issues when caring for someone with MND (Ng et. al 2011). This finding in particular has a detrimental effect on the wellbeing (mental & physical) of the carer. The level of care they can provide diminishes affecting the wider family group as well as attitudes towards health professionals.
The primary aim of this project is to improve carer wellbeing. This will be achieved by developing and piloting a unique mental health support plan sensitive to the perceived emotional demands of family carers of people with MND. The tool will enable the user (eg. the carer) to recognise mental health issues in themselves and prompt strategies to mitigate these issues in a timely manner. In the future a similar support plan can be developed for the person with MND.
Mental health plans are implemented in mental health care in the United Kingdom. A full literature search of mental health plans has taken place. Although Borderline Personality Disorder (BPD) is a very different condition to MND its' support plan is based on a series of symptoms or feelings the person may be experiencing. The framework of this plan has been used to underpin the foundation of the MND Carer Health plan in particular the psychological support that carers of pwMND need.
The short questionnaire and support plan is completed during carer interview and is a dynamic document which can be revised as situations change.
It is anticipated that successful implementation of this support plan will:
• reduce worry
• reduce stress & distress for carers during the duration of the disease
• reduce the economic impact attached to carer burden as evidenced by Sue Ryder (2018)
Funding for this project has been awarded by the Gordon Aikman Scholarship Scheme.
References : Ng, L.., et al. (2011), Patient/carer perceptions of disability in motor neurone disease and carer coping, International Journal of rehabilitation Therapy,18(10)pp.568-578. Radakovic, R. (2018), The Brief Dimensional Apathy Scale (b-DAS): a short clinical assessment of apathy.

1995: **Ocular system in Amyotrophic Lateral Sclerosis patients and relation with bulbar function**.
Federica Cozza* (1, 2), Andrea Lizio (1), Valeria Ada Sansone (1, 3), Christian Lunetta (1).
1. Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milan, Italy.
2. Department of Materials Science & Optics and Optometry Research Center (COMiB), University of Milano-Bicocca, Milan, Italy.
3. Department of Biomedical Sciences of Health, University of Milan, Milan, Italy.
Background: During later stages of Amyotrophic Lateral Sclerosis (ALS), having clear vision and functional ocular motility is one of a key aspect for the patient who usually communicate with eye-tracking system. The visual system is generally preserved in ALS patients, but some signs of eye dysfunction have been detected and reported in recent papers, in particular related to bulbar function. The aim of this study is to perform some optometric tests on ALS patients and evaluate the relation between the ocular findings and level of bulbar impairment.
Patients and Methods: Patients with definite, probable and probable laboratory supported ALS were involved in the study. The optometric protocol included an ocular history and symptoms questionnaire, extraocular motility test (EOM test), the Northeastern State

University College of Optometry (NSUCO) oculomotor test, near point of convergence (NPC), error refraction measurement, visual acuity, heterophoria and heterotropia assessment. The relation between the optometric tests and the bulbar domain of ALS Functional Rating Scale - revised (ALSFRS-r) was investigated using the Spearman correlation coefficient and the Wilcoxon rank-sum test as appropriate.
Results: We recruited 96 ALS patients with median age 64.00 [52.50-72.00] years and M/F ratio of 1.46 (M/F: 57/39). The median disease duration was 40.72 [22.40-83.73] months. The optometric analysis showed the presence of significant ocular symptoms in 73% of the patients. In detail, we found a relation between the severity of bulbar function measured by bulbar domain of ALSFRS-r and eyestrain (p=0.0023), burning eye sensation (p=0.0480) and photophobia (p=0.0052). Moreover, the patients who showed slowed eye movements in EOM test reported significantly lower bulbar domain score (p=0.0194). Considering the NSUCO protocol, the increasing in accuracy score was related to a significant increase in bulbar domain score, in both saccades and pursuits tests (p=0.0212; p=0.0440 respectively). Also the NPC test revealed that the lower the value of bulbar domain, the worse was the performance in convergence assessment (p=0.0017).
Conclusions: Our results showed that some aspects of the oculomotor function in ALS patients are related to the bulbar district, consistently with the literature. The assessment of these ocular parameters becomes essential for clinicians, since patients with bulbar impairment are those who often use eye-gaze communication.

2003: **A retrospective service analysis of needle electromyography referrals to the King's College Hospital Neurophysiology Department.**

Thomas Weddell(*)(1), James Bashford (2), Christopher Shaw(2)(3)(4)
1. King's College Hospital NHS Foundation Trust, London, UK.
2. Wohl Clinical Neuroscience Institute King's College London, King's College Hospital NHS Foundation Trust, London, UK.
3. UK Dementia Research Institute, London, UK.
4. Institute of Psychiatry, Psychology and Neuroscience, King's College London and King's Health Partners, London, UK.

Introduction: Needle electromyography (NEMG) is the standard neurophysiological technique to aid the diagnosis of amyotrophic lateral sclerosis (ALS). NEMG can be painful for patients and often requires long distance travel to specialist centres. We have recently developed a non-invasive, high-density surface EMG (HDSEMG) for research purposes (SPiQE), which accurately identifies fasciculations, an early hallmark of ALS. However, there were high withdrawal rates (45%) in our 12-months prospective study of 25 ALS patients due to worsening disability. It became clear that a portable device for at home use would be invaluable in a research setting.
To gauge portable HDSEMGs additional clinical utility we performed a retrospective service evaluation of NEMG referrals to King's College Hospital (KCH) Neurophysiology Department. We reviewed the diagnostic burden in relation to fasciculations and ALS on the department and their patients.
Methods: We used a departmental database to identify all NEMG referrals made over 6 months, Jan - Jun 2017. Hospital records allowed us to identify patient referrals querying ALS or with fasciculations as the presenting symptom and their NEMG reports.
Results: A total of 1424 NEMG referrals were received. Of these, 96 (6.7%) met our inclusion criteria, 90 outpatients and 6 inpatients. Outpatient findings of note included:
• Median waiting time from referral to testing was 34 days (IQR 12)
• Median return travel distance by road to appointments was 46km (IQR 83). Median travel time by car was 95 mins (IQR 57.5) and by public transport was 139 mins (IQR 81)

• 26 were diagnosed with ALS (29%) and 85% of those were positive for fasciculations. Remaining referral diagnoses: BFS 7.8%, normal 42%, MG 1.1%, spinal/radicular 8.9%, neuropathy 5.6%, inconclusive 5.6%
• 18 repeat tests were required amongst 15/90 (16.7%) patients.
Conclusions: We have evaluated the referrals for NEMG at KCH for ALS and/or fasciculations. Given the physical burden placed on patients attending specialist centres, portable HDSEMG has potential clinical utility for community screening. Further study of its cost-effectiveness and practicability is required. Sufficient calibration may allow this tool to be used to identify 'red flag' features earlier in a subset of referrals and prompt more focused diagnosis with conventional techniques. Diagnosis of ALS at an earlier stage is a key objective, leading to earlier enrolment in clinical trials.

2004: **The prognostic value of a brief respiratory follow-up in ALS.**

Alice Pirola (1)*, Elisa De Mattia (1), Andrea Lizio (1), Fabrizio Rao (1), Giulia Sannicolò (2), Valeria Sansone (1,3), Christian Lunetta (1).
(1) NEuroMuscular Omnicentre, Fondazione Serena Onlus, Milan, Italy.
(2) U.O. NPI, A.P.S.S, Trento, Italy.
(3) Dept. Biomedical Sciences of Health, University of Milan.

Background: Amyotrophic Lateral Sclerosis (ALS) patients suffer progressive respiratory muscle weakness, leading to ventilatory failure and ineffective cough, principal causes of morbidity and mortality. The regular monitoring of respiratory functions is therefore of great importance. Our aim was to evaluate the relationship among the median monthly decline of different routinely performed respiratory measures during a 6-months follow-up period and survival.
Patients and Methods: ALS patients referred to our centre between January 2012 and December 2017, with respiratory assessments performed at baseline and six months later, were included. Forced vital capacity, in seated and supine position (FVC; sFVC), and peak expiratory cough flow (PCEF) were assessed. Monthly declines were calculated for each respiratory assessment.
Results: We included 73 ALS patients with mean age of 62.44 ± 9.93 years and a male/female ratio of 2.48 (M/F: 52/21). Considering the monthly decline as a continuous outcome, Cox model showed that both FVC% and sFVC% were significantly associated with survival (HR=0.885; p=0.0445 and HR=0.892; p=0.0266, respectively). These relations remained significant using the same variables as dichotomous (log rank p=0.0022 for FVC%; log rank p=0.0371 for sFVC%), considering the cut-off of -3% /month for FVC% and -1% /month for sFVC%, calculated using an outcome-oriented approach based on the log-rank test statistic. Moreover, the relationship with survival of both FVC% and sFVC% suggested a two-ways interaction. Therefore, FVC% and sFVC% were categorized using the respectively cut-offs into one hybrid variable with four levels, which showed a significant difference in survival. Specifically, the worst prognosis in terms of survival was found in patients with both FVC% and sFVC% below the respective cutoffs.
Conclusions: Our study confirmed the measure of vital capacity holds significant survival prognostic value. However, although previous works evidenced a higher sensitivity of sFVC% rate of decline compared to FVC% in predicting 2-year survival (Baumann et al., 2010), our results, combining the same two respiratory variables into a hybrid one, showed a stronger power to predict the prognosis, particularly when both FVC% and sFVC% were below their cut-off value. Our results reinforced the importance of having the measurement of (forced) vital capacity performed both in seated and supine positions.

2014: **A patient handbook for psychological support**.

Dorotohée Lulé (1), Magdalena Kuźma-Kozakiewicz (2,3), K. Barc (2), A. C. Ludolph (1), P. Andersen (4).
1 Department of Neurology, University of Ulm, Ulm, Germany
2 Department of Neurology, Medical University of Warsaw, Warsaw, Poland
3 Neurodegenerative Diseases Research Group, Medical University of Warsaw, Warsaw, Poland
4 Institute of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden.

Background: For the patients and their families the psychosocial impact of the diagnosis is extraordinary, most feel lost and desperate. The consulting physician is the person to communicate the diagnosis and to provide first aid with regard to psychosocial support. Yet, patients and their families are overwhelmed and are unable to address all questions which come up to their mind in the first place. For clinical decision making, there have been multiple guidelines when to choose which kind of therapeutic measure in the course of ALS. Yet, there is a major lack of standardised guidelines on how to deal with this psychological impact and how to provide psychological support beyond what the treating physician and other members of the multidisciplinary care teams are capable to provide during consultation.
Methods: We developed a guidebook for psychological support using major findings on psychosocial adjustment and therapy from our JPND funded project NEEDSinALS (www.NEEDSinALS.com), from the literature and clinical routine. These findings are categorized within 11 chapter, which address questions and concerns which have been mentioned by ALS patients and their family members.
Results: Among the 11 chapters of the guidebook, topics such as psychosocial impact of ALS, factors to support psychosocial adaptation including social support, cognition in ALS, impact on family members and information on false friends are listed. Furthermore, most important links and addresses for patient organizations, social services and networks are mentioned.
Discussion: Experience has supported the idea that there is a great need for information on ALS which goes beyond medical support alone. The hereby presented handbook is the first to our knowledge to provide information on how to facilitate and support psychosocial adaptation in ALS in a standardised way. A guidebook can never substitute personal communication by interdisciplinary professionals on ALS. However, the guidebook might add to the valuable work which can be provided within clinical routine.

2017: **Prognostic value of weight loss in patients with amyotrophic lateral sclerosis (ALS).**

Mark R. Janse van Mantgem (1)*, Ruben P.A. van Eijk (1,2), Henk-Jan Westeneng (1), Leonard H. van den Berg (1).
1. Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands.
2. Biostatistics & Research Support, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands.

Objective: The aim of this study was to determine the independent prognostic value of weight loss (WL) prior to diagnosis in patients with ALS.
Methods: Analyses were performed with total and monthly absolute and relative WL and categorized according to severity. Cox proportional hazard models were used to assess its prognostic value. All models were adjusted for the predicted prognosis based on progression rate, diagnostic delay, age at onset, forced vital capacity, bulbar onset, definite ALS, presence of frontotemporal dementia and C9orf72. Missing data were handled by multiple imputations.

Results: The dataset comprised of 2,420 consecutive patients diagnosed with ALS between 2010 and 2018. WL was reported in 67.8 % of the patients: 74.1% in bulbar onset patients and 63.3% in spinal onset patients. Bulbar onset patients had relatively more WL as compared to spinal onset patients (7.1% vs 5.5% respectively of their boy mass, $p < 0.001$). WL was a strong independent predictor of survival ($p < 0.001$), with a dose-response relationship between the relative amount of WL and risk of death: HR 0% vs <5% 1.11, 95% CI 0.93 – 1.32, $p = 0.25$; HR 0% vs 5-10% 1.32, 95% CI 1.12 – 1.55, p 10% 1.81, 95% CI 1.53 – 2.13, p 0.4), or genetic subgroups as C9orf72 and UNC13A and cognitive markers ($p > 0.05$).
Conclusions: This study shows that WL at time of diagnosis is a strong independent predictor of survival in ALS patients. This knowledge is important for clinicians to make more accurate predictions when counseling patients, for prioritizing patients for dietary interventions or as selection criterion for clinical trials.

2046: **Emotional lability at disease onset is a prognostic factor of faster disease progression in amyotrophic lateral sclerosis**.

Krzysztof Barc (1*), Krzysztof Nieporecki (1), Katarzyna Szacka (1, 2), Mamede de Carvalho (3), Marta Gromicho (3), Julian Grosskreutz (4), Susanne Petri (5), Annekathrin Rödiger (4), Robert Steinbach (4), Hilmi Uysal (6), Magdalena Kuzma-Kozakiewicz (1, 2).
(1) Department of Neurology, University Clinical Centre of the Medical University of Warsaw, Warsaw, Poland
(2) Department of Neurology, Medical University of Warsaw, Warsaw, Poland
(3) Faculdade de Medicina-IMM, Universidade de Lisboa, Lisbon, Portugal
(4) Hans-Berger Department of Neurology, Jena University Hospital, Germany
(5) Department of Neurology, Hannover Medical School, Hannover, Germany
(6) Department of Neurology, Akdeniz University Faculty of Medicine, Antalya, Turkey

Background: Beside motor disability, patients with ALS experience a range of non-motor symptoms, including emotional lability (EL), known as pseudobulbar affect. It is either present at the disease onset or develops with time causing severe distress to patients and their proxy.
The aim of the study was to assess the impact of EL at ALS onset on disease progression.
Methods: The analysis included 1333 patients from Poland, Germany, Portugal and Turkey (ONWebDUALS). The mean age at onset was 59.3±13.7 with M:F ratio of 1.36. Emotional lability at ALS onset was present in 16.05% patients. The disease prognosis was evaluated with ALSFRS-R decline rate (48–ALSFRS-R/disease duration). The results were analysed with respect to age, gender, bulbar/limb onset, cognitive impairment at onset and the use of anti-depressive drugs. The statistical analysis included Mann–Whitney U-test, multiple two-way analysis of variance (ANOVA) and Chi-square test.
Results: EL at ALS onset resulted in a significantly higher ALSFRS-R decline rate as compared to patients without EL (0.68 vs 0.48, $p<0.0001$). It was found in both bulbar (0.79 vs 0.65, $p<0.05$) and limb onset ALS (0.55 vs 0.43, $p<0.001$). In patients with EL at onset, there were significant differences in ALSFRS-R bulbar (0.19 vs 0.04, $p<0.001$), motor (only when limb onset, 0.41 vs 0.35, $p<0.05$) and respiratory subscores (0.02 vs 0.00, $p<0.005$) as compared to patients without EL. The bulbar onset and female gender were associated with faster ALSFRS-R decline ($p<0.0001$) and were more often present in the group with EL (43.93% vs 21.89%, $p<0.0001$ and 50.00% vs 40.84%, $p<0.05$, respectively). To exclude a direct influence of bulbar onset and female gender on the observed results, the two-way analysis of variance (ANOVA) was applied showing no significant interactions. Although cognitive impairment at ALS onset was also more often present in patients experiencing concomitant EL (30.37% vs 6.7%, $p<0.0001$), it did not influence ALSFRS-R decline rate. Interestingly, we found a trend showing that provision of anti-depressive treatment in

patients with EL at onset was associated with a slower ALSFRS-R decline rate (0.53 vs 0.70, p=0.12).
Conclusions: Emotional lability at disease onset was found to be an independent predictor factor of faster disease progression in ALS.
Acknowledgements: This study was supported by EU Joint Programme – Neurodegenerative Disease Research (JPND) project: OnWebDuals (DZP/2/JPND-III/2015).

2054: **The clinical profile of primary lateral sclerosis: a population-based study**.
Eoin Finegan*(1), Rangariroyashe H Chipika(1), Stacey Li Hi Shing(1), Colette Donaghy(1), Orla Hardiman(1), Peter Bede(1).
Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin

Background: Primary lateral sclerosis (PLS) is a progressive upper motor neuron disorder. The diagnosis of PLS is one of exclusion and requires a period of 4 years without evidence of lower motor neuron involvement. No validated diagnostic or prognostic biomarkers exist for suspected PLS patients.
Objectives and Methods: This population-based, prospective study aimed to evaluate a large cohort of PLS patients using standardised clinical measures including the ALSFRS-R, manual muscle-strength testing, UMN-burden scores, tapping rates and spasticity assessment.
Results: 36 patients with established PLS were enrolled. Lower limb symptom onset was reported in 97% of patients, in marked contrast to the variability of symptom onset in ALS. No PLS patient developed their initial symptom in the upper limbs though all 36 had clinical UMN signs in the upper limbs at the time of assessment. Lower limb function (ALSFRS-r M=5.39, SD 1.57) was significantly (p<0.001) more impaired than upper limb function (ALSFRS-r M=8.64, SD 2.00). Motor disability was less severe in the upper limbs than in the lower limbs in all patients. Pseudobulbar impairment was present in 78% of the patients and respiratory function was well-preserved in the entire group. A symmetrical pattern of spasticity was detected between the right (M=3.10, SD 0.75) and left sides (M=3.22, SD 0.72), p=0.10. Spasticity was significantly greater lower limbs (Mean=3.53, SD 0.77) than in the upper limbs (Mean=2.88, SD 0.97), p<.001. UMN-burden limb sub-scores negatively correlated with finger tapping rates in the corresponding upper limb (p<.001). Foot-tapping rates were associated with reduced lower limb functional scores; (p=.001) and finger-tapping rates were also strongly associated with reduced upper limb functional sub-scores (p=.001).
Conclusions: Contrary to ALS, PLS patients exhibit strikingly homogeneous patterns of disability and disease propagation. The identification of PLS-associated clinical signatures may facilitate an earlier diagnosis of suspected cases which is highly relevant given the considerably longer survival in PLS compared to ALS.

2055: **Behavioral assessment of attention in ALS.**
Maher Zoubi *(1,2), Marcel Daamen (2), Patrick Weydt (1,2), Xenia Kobeleva (2,3).
1 University Hospital Bonn, Department of Neurodegenerative Diseases and Gerontopsychiatry, Bonn, Germany.
2 Clinical Research, DZNE Bonn, Bonn, Germany.
3 University Hospital Bonn, Department of Neurology, Bonn, Germany.

Background: Due to an overlap between ALS and frontotemporal dementia up to 50% of ALS patients exhibit cognitive impairment (Rippon et al. 2006). We aimed to evaluate if ALS patients without clinical evidence of cognitive dysfunction would show behavioral atten-tional deficits performing a modified version of the Attention Network Test (ANT) (Fan et al. 2005).

Methods: 23 ALS patients and 25 matched controls took part in the behavioral study and underwent a neuropsychological screening using ECAS (Abrahams et al. 2014). In a modified version of the ANT (Firbank et al. 2016), the subjects pressed a button in the direction of the majority of four arrowheads which were either congruent (all arrows in one direc-tion) or incongruent (all but one in the same direction, two levels of difficulty). Participants completed 120 trials, which were preceded by a cue in 50% of the cases (alerting). Statistical analysis included a comparison of absolute reaction times and reaction time differences such as executive effect (incongruent - congruent), conflict effect (incongruent hard - incongruent easy) and alerting effect (no cue - cue), and of ECAS performance.
Results: In terms of absolute reaction times, patients consistently showed longer reaction times than controls. However, when comparing relative reaction time differences (executive, conflict or alerting effect) patients performed equally well as controls (all p-values > 0.05). The results of the ECAS in our study cohort were comparable between groups (all p-values > 0.05).
Discussion: To account for motor impairment in ALS we focused on relative instead of absolute reaction times. We found intact alerting, executive and conflict effects in ALS, as indicated by a modulation of the reaction times by task condition. However, we did not find significant differences of attentional-executive performance between groups. Neither did we observe differences of cognitive performance in the ECAS. These results were surprising, given the large amount of evidence of frontoparietal dysfunction (i.e. Xu et al. 2017). We assume the absence of any difference in attentional and neuropsychological performance between groups is mainly due to a selection bias of cognitively intact patients, a small sample size and possibly also due to methodological issues such as a lack of sensitivity of the task. Future work will concentrate on inclusion of cognitively impaired patients as well as on the association with clinical symptoms.

2056: **Empathy in ALS patients and their next of kin: a pilot study.**

A Martínez* (1,4), L. Serrano Pastor (2), A. Gomila (2), J.F. Vázquez Costa (3,4,5).
1. CARENA Association. Fundació Obra Social "La Caixa"
2. Psychology, Evolution and Cognition (IFISC-CSIC), University of the Balearic Islands, Palma, Spain
3. Neuromuscular Research Unit, Instituto de Investigación Sanitaria la Fe (IIS La Fe), Valencia, Spain.
4. ALS Unit, Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain.
5. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain.

Introduction: A variable degree of cognitive and/or behavioral impairment have been described in up to 50% of ALS patients. Recently, deficits in the social cognition and theory of mind (ToM) have been incorporated to the cognitive profile of ALS patients. Empathy relates to affective ToM, and changes in empathy, could influence the decision-making process and the quality of life of patients and their carers. However, little is known about empathy in ALS patients and their next of kin.
Objective: To describe empathy in a cohort of ALS patients and their next of kin.
Methods: 20 patients and 20 relatives were included in this pilot study. All were cognitively studied with the ECAS. Behavioral changes in ALS patients were assessed by their relatives with the semi-structured behavior interview of ECAS and the FRSBE questionnaire. ALS patients were classified as having cognitive and/or behavioral impairment according to the current criteria. For the empathy assessment the Interpersonal Reactivity Index (IRI), which includes four subscales (Perspective-Taking, Fantasy, Empathic Concern and Personal Distress) was administered. Normative values were used to calculate Z-scores.
Results: Eleven ALS patients were classified as having cognitive and/or behavioral impairment according to the current criteria. ALS patients showed only a slightly reduced fantasy compared to their relatives (Z-score: -0.6 vs -0.2). Five ALS patients (20%) vs six

relatives (30%) scored below the more than 2SD lower than the normative values in any of the empathy items, although they differ in ALS patients and controls. No association between empathy and cognitive or behavioral scores was found.
Conclusions: In this pilot study, a mild reduction in the fantasy score of empathy were found in ALS patients vs their relatives. The association of these changes with other cognitive or behavioral changes and their influence in the decision making process, should be addressed in future studies.

2057: **Facial onset sensory and motor neuronopathy: a motor neuron disease with an oligogenic origin?**

J.F. Vázquez-Costa (1,2,3), L. Pedrola (4), S. Moreau-Le Lan (4), I. Teresí-Copoví (5), M. Frasquet (1,2,3), MJ Chumillas (1,3,5), T Sevilla (1,2,3,6).
1. Neuromuscular Research Unit, Instituto de Investigación Sanitaria la Fe (IIS La Fe), Valencia, Spain.
2. ALS Unit, Department of Neurology, Hospital Universitario y Politécnico La Fe, Valencia, Spain.
3. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain.
4. Genomic Unit, Instituto de Investigación Sanitaria La Fe, Valencia, Spain.
5. ALS Unit, Department of Neurophysiology, Hospital Universitario y Politécnico La Fe, Valencia, Spain.
6. Department of Medicine, University of Valencia, Valencia, Spain.

Objective: To describe a patient with facial onset sensory and motor neuronopathy (FOSMN) carrying heterozygous mutations in both TARDBP and SQSTM1 genes.
Methods: The patient underwent neurological, neuropsychological and neurophysiological examinations. Brain magnetic resonance imaging (MRI) and extensive genetic analysis were also performed.
Results: The neurological examination showed dysphonia, left trigeminal hypoesthesia, and left masseter and temporalis muscle atrophy. Mild cognitive impairment, affecting predominantly executive functions and social cognition, was appreciable in the neuropsychological examination. The electrophysiological studies revealed: left abnormal blink reflex; neurogenic changes in bulbar and cervical muscles; normal motor evoked potential amplitude, central motor conduction time and cortical silent period. Brain MRI showed right-predominant fronto-temporal atrophy. Genetic analysis showed a heterozygous mutation in TARDBP (p.A390S) and in SQSTM1 (p.P392L), both previously described as causing amyotrophic lateral sclerosis. The SQSTM1, but not the TARDBP, mutation was found in both healthy siblings.
Conclusions: Our data provide new clinical, neuroimaging and genetic evidence that FOSMN is a neurodegenerative disease of the motor neuron disease and frontotemporal dementia spectrum, with a possible oligogenic origin. Multicentric efforts focusing on cognitive and genetic studies are necessary to confirm this hypothesis and to determine if ALS genes should be systematically screened in these patients.

2060: **Posterior cerebral changes in ALS: Occipital and parietal pathology**.

Rangariroyashe Hannah Chipika* (1), Eoin Finegan (1), Stacey Li Hi Shing (1), Orla Hardiman (1), Peter Bede (1).
(1) Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.

Background: ALS is widely recognised as multi-system disorder with extensive motor and extra-motor involvement. The objective of this study is the multiparametric characterisation of posterior cerebral pathology in ALS.

Methods: Eighty-five patients and 76 healthy controls were enrolled into a prospective neuroimaging study. Whole-brain and region of interest grey and white matter analyses were performed assessing regional brain volumes and indices of white matter integrity; fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD).
Results: Whole brain analyses revealed extensive extra-motor grey and white matter pathology. Region of interest analyses highlighted additional grey matter volume reductions in parietal and occipital areas. Significantly FA reductions and increased RD were observed in both occipital and parietal regions whereas increased AD was detected in the parietal lobes.
Conclusions: Extra-motor pathology in ALS is not confined to frontal and temporal regions, but includes occipital and parietal brain regions. The imaging profile of extra-motor regions is consistent with recent neuropathology studies. The characterisation of extra-motor involvement in ALS has implications for individualised patient care, caregiver support and the development of novel biomarkers.

2061: **Cognitive impairment spreading across clinical stages in patients with amyotrophic lateral sclerosis.**

*Umberto Manera (1), Cristina Moglia (1,2), Antonio Canosa (1), Rosario Vasta (1), Maura Brunetti (1), Marco Barberis (1), Lucia Corrado (4), Sandra D'Alfonso (4), Enrica Bersano (5), Maria Francesca Sarnelli (5), Valentina Solara (5), Jean Pierre Zucchetti (1), Laura Peotta (1), Barbara Iazzolino (1), Letizia Mazzini (5), Gabriele Mora (6), Andrea Calvo (1,2), Adriano Chiò (1,2,3).
(1) ALS Center, 'Rita Levi Montalcini' Department of Neuroscience, University of Torino, Turin, Italy.
(2) Azienda Ospedaliero-Universitaria Città della Salute e della Scienza of Torino, Turin, Italy.
(3) Institute of Cognitive Sciences and Technologies, National Research Council, Rome, Italy.
(4) Department of Health Sciences Interdisciplinary Research Center of Autoimmune Diseases, "Amedeo Avogadro" University of Eastern Piedmont, Novara, Italy.
(5) ALS Center, Department of Neurology, Azienda Ospedaliera Universitaria Maggiore della Carità, Novara, Italy.
(6) Istituti Clinici Scientifici Maugeri, IRCCS Milano, Italy.

Introduction: In Amyotrophic lateral sclerosis (ALS) the cognitive domains involvement is detectable in 50% of all cases. A recent study performed on a cross-sectional clinical-based cohort has pointed out that cognitive and behavioural deficits tends to be more frequent and severe with advanced disease.
Objective. To assess the association of the degree of severity of motor impairment, classified using King's and MiToS ALS staging systems, to degree of cognitive impairment in a large cohort of ALS patients.
Methods: This is a population-based cross-sectional study on ALS patients incident in Piemonte, Italy, between 2007 and 2015. ALS patients underwent a complete battery of neuropsychological tests encompassing executive function, memory, visuospatial function, social cognition and language, selected according to the Diagnostic Criteria for the Behavioural variant of Frontotemporal Dementia13 and the ALS-FTD Consensus Criteria. Both King's and the Milano Torino Staging (MiToS) systems were used for defining the severity of motor impairment.
Results: Of the 797 patients included in the study, 163 (20.5%) with ALS-FTD, 38 (4.8%) with cognitive and behavioral impairment (ALScbi), 132 (16.6%) with cognitive impairment (ALSci), 63 (7.9%) with with behavioral impairment (ALSbi), 16 (2.0%) with non-executive impairment, and 385 (48.2%) cognitively normal. According to King's staging, the frequency of cases with ALSFTD progressively increased from 16.5% in stage 1 to 44.4% in stage 4; conversely the frequency of ALSci, ALSbi and ALScbi increased from King's stage 1 to King's stage 3 and decreased thereafter. A similar pattern was observed with the MiToS staging; the frequency of ALS-FTD increased from 20.2% in MiToS stage 0 to 50.0% in

stage 2. Overall, cognitive impairment became much more frequent as patients disease progressed based on both the King's and MiToS staging systems. ALS-FTD was more frequent in patients with bulbar involvement at time of cognitive testing. Patients with C9ORF72 expansion (n=61) showed more severe cognitive impairment with increasing both King's and MiToS stages.
Conclusion: Our findings suggest that ALS motor and cognitive components may worsens in parallel over time, and that cognitive worsening becomes more pronounced when bulbar function is involved. Our data support the hypothesis that ALS pathology disseminates in a regional ordered sequence, through a cortico-efferent spreading model.

2065: **Date of Onset as an Indicator and Predictor of Data Quality in Clinical Research.**

Ervin Sinani(1), Alexander Sherman(1)*
Neurological Clinical Research Institute, Massachusetts General Hospital, Boston, USA.

Objective: Establish reliability and credibility of Disease Duration (DxD) as a major criterion for patient eligibility in clinical trials and as a major component in several predictive and staging models
Background: Date of Onset (DoO) and its immediate derivative, DxD play major role as predictors of patients' disease progression and as a major indicator of patients' eligibility in Randomized Clinical Trials (RCT). While analyzing and harmonizing multiple datasets, some of which may contain records for same individuals, we noticed an interesting phenomenon: values in DoO field are more precise in RTC datasets than in observational studies, as most RTCs require DxD values as a precondition for patients' enrollment. While acknowledging an imprecise nature of patients' recollections of first symptom onset dates, as well as Investigators' overconfidence in their abilities to trigger patients' memories, we question the utility of DxD values in predictive (Origent's, delta50, etc.) and staging models in ALS/MND as well as a major eligibility criterion in RCTs.
Design/Methods: We analyzed 33 datasets from RCT and biomarker studies, mostly from NEALS ALS consortium-led projects, and clustered patient records (3123) based on degrees of precision of DoO field values as follows:
- complete date known (day/month/year)
- day unknown (UU/month/year), or
- day/month unknown (UU/UU/year).
We found some subjects, for whom, with a high degree of probability we were able to locate matching records in multiple datasets, which allowed us to compare corresponding DoO values.
Results: Based on percentage of volunteers for whom day and month of DoO were unknown, we were able, with exception of one RCT, to predict whether records are coming from RCTs (0-2%) or biomarker studies (4-14%).
For those research volunteers with records in multiple datasets, we found the same trend – while in observational studies day and month may be unknown, in RTC datasets the values were almost always precise.
Conclusions: While DxD is essential and plays a major role in disease models and trials' eligibility, we question its reliability and objectivity. Perhaps, a more reliable date, Date of Diagnosis in combination with known on that date ALSFRS-R and El Escorial values would be a better predictor and shall be utilized instead.
Acknowledgements: We thank Investigators and member-sites' personnel of NEALS ALS, PRO-ACT, PRO-ACE, and ALS/MND Natural History consortia.

2066: **Increased resting energy expenditure compared to predictive theoretical equations in Amyotrophic Lateral Sclerosis: a study versus control.**

Pierre Jésus(1,2,3)*, Philippe Fayemendy(1,2,3), Benoit Marin(2,3,4), Marie Nicol(2,3,5), Huguette Sourisseau(1), Yves Boirie(6,7), Stéphane Walrand(7), Najate Achamrah(8,9), Moïse Coëffier(8,9), Pierre-Marie Preux(2,3,4), Géraldine Lautrette(5), Philippe Couratier(2,3,5), Jean-Claude Desport(1,2,3).
1 Nutrition Unit, University Hospital of Limoges, Limoges, France.
2 INSERM, U1094, Tropical Neuroepidemiology, Limoges, France.
3 Univ. Limoges, UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, CNRS FR 3503 GEIST, Limoges, France.
4 Center of Epidemiology, Biostatistics and Research Methodology (CEBIMER), University Hospital of Limoges, Limoges, France.
5 ALS center, University Hospital of Limoges, Limoges, France.
6 Université Clermont Auvergne, CHU Clermont-Ferrand, Service de Nutrition Clinique, CRNH Auvergne, Clermont-Ferrand, France
7 Université Clermont Auvergne, INRA, UNH, Unité de Nutrition Humaine, CRNH Auvergne, Clermont-Ferrand, France
8 Nutrition Department, Clinical Investigation Centre CIC-1404, University Hospital, Rouen, France.
9 INSERM UMR 1073, Nutrition, Inflammation and Gut-Brain axis dysfunction, Normandie University, UNIROUEN, Rouen, France.

Introduction: About 50-60% of Amyotrophic Lateral Sclerosis (ALS) is characterized by hypermetabolism, defined as 10% or more excess resting energy expenditure (REE) compared to theoretical values. Harris and Benedict's (HB) formula is mainly used to predict REE, but others are also applied in current practice. The aims of this study were i) to assess hypermetabolism in ALS patients compared to a control population ii) to assess survival in patients with or without major hypermetabolism (REE variation > 20%), according to different REE formulas.
Method: Nutritional assessments were performed in ALS patients and in healthy control population. REE was measured (mREE) by indirect calorimetry and calculated (cREE) using HB 1919, Mifflin, Owen, Wang and Rosenbaum formulas. We studied survival with Log-rank test.
Results: 315 ALS patients and 80 controls were included. mREE was higher in ALS patients than cREE with all formulas ($p < 0.0001$) and higher versus controls ($p = 0.0002$). Depending on the predictive equation, hypermetabolism was found in 35.2% to 73.7% of ALS patients and higher versus controls (p 20%) was found in 10.2% to 44.4% of ALS patients versus 0% to 20.0% in controls (p 20% had a lower survival ($p = 0.01$ and $p = 0.002$ respectively).
Conclusion: Hypermetabolism is present according to the different REE predictive equations used and higher than in controls. In clinical practice REE formulas such as HB 1919 or Mifflin can be used as a reference value compared to IC to diagnose hypermetabolism in ALS.

2069**: Resting energy expenditure equations in Amyotrophic Lateral Sclerosis, creation of an ALS-specific equation**.

Pierre Jésus (1,2,3)*, Benoit Marin (2,3,4), Philippe Fayemendy (1,2,3), Marie Nicol (2,3,5), Géraldine Lautrette (5), Huguette Sourisseau (1), Pierre-Marie Preux (2,3,4), Philippe Couratier (2,3,5), Jean-Claude Desport (1,2,3).
1 Nutrition Unit, University Hospital of Limoges, 87000 Limoges, France.
2INSERM, U1094, Tropical Neuroepidemiology, 87000 Limoges, France.
3Univ. Limoges, UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, CNRS FR 3503 GEIST, F-87000 Limoges, France.
4 Center of Epidemiology, Biostatistics and Research Methodology (CEBIMER), University Hospital of Limoges, 87000 Limoges, France.

5 ALS center, University Hospital of Limoges, 87000 Limoges, France.

Introduction: Resting energy expenditure (REE) formulas for healthy people (HP) are used to calculate REE (cREE) in amyotrophic lateral sclerosis (ALS) patients. In 50-60% of ALS cases an increase of measured REE (mREE) in indirect calometry (IC) compared to cREE is found. The aims here were (i) to assess the accuracy of cREE assessed using 11 formulas as compared to mREE and (ii) to create (if necessary) a specific cREE formula for ALS patients.
Method: 315 Patients followed in the ALS expert center of Limoges between 1996 and 2014 were included. mREE assessed with IC and cREE calculated with 11 predictive formulas (Harris Benedict (HB) 1919, HB 1984, WSchofield, De Lorenzo, Johnstone, Mifflin, WHO/FAO, Owen, Fleisch, Wang and Rosenbaum) were determined at the time of diagnosis. Fat free mass (FFM) and fat mass (FM) were measured with impedancemetry. A Bland and Altman analysis was carried out. The percentage of accurate prediction ± 10% of mREE, and intraclass correlation coefficients (ICC) were calculated. Using a derivation sample, a new REE formula was created using multiple linear regression according to sex, age, FFM and FM. Accuracy of this formula was assessed in a validation sample.
Results: ICC ranged between 0.60 and 0.71 (moderate agreement), and percentage of accurate prediction between 27.3% and 57.5%. Underestimation was found from 31.7% to 71.4% of cases. According to these unsatisfactory results we created an ALS-specific formula in a derivation sample (130 patients). ICC and percentage of accurate prediction increased in a validation sample (143 patients) to 0.85 (very good agreement) and 65.0% respectively, with 17.5% underestimation.
Conclusion: REE formulas for HP underestimate REE in ALS patients compared to mREE. Our new ALS-specific formula produced better results than formulas for HP. This formula can be used to estimate REE in ALS patients if IC is not accessible.

2070: **Resting energy expenditure is increased in ALS patients when compared to healthy subjects**.

Philippe Fayemendy (1,2,3), Benoit Marin (2,3,4), Marie Nicol (2,3,5), Anaïs Labrunie (2,4), Huguette Sourisseau (1), Yves Boirie (6,7), Stéphane Walrand (7), Najate Achamrah (8,9), Moïse Coëffier (8,9), Pierre-Marie Preux (2,3,4), Géraldine Lautrette (5), Jean-Claude Desport (1,2,3), Philippe Couratier (2,3,5), Pierre Jésus(1,2,3)*.
1 Nutrition Unit, University Hospital of Limoges, Limoges, France. (PJ, PF, HS, JCD)
2INSERM, U1094, Tropical Neuroepidemiology, Limoges, France.
3Univ. Limoges, UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, CNRS FR 3503 GEIST, F-87000 Limoges, France.
4 ALS center, University Hospital of Limoges, France.
5 Center for Epidemiology, Biostatistics and Methodology of Research, University Hospital of Limoges, France
6 University Clermont Auvergne, University Hospital of Clermont-Ferrand, Clinical Nutrition Department, CRNH Auvergne, F-63000 Clermont-Ferrand, France
7 University Clermont Auvergne, INRA, UNH, Human Nutrition Unit, CRNH Auvergne, F-63000 Clermont-Ferrand, France
8 Nutrition Department, Clinical Investigation Centre CIC-1404, University Hospital, Rouen, France.
9 INSERM UMR 1073, Nutrition, Inflammation and Gut-Brain axis dysfunction, Normandie University, UNIROUEN, Rouen, France.

Introduction: The increase of resting energy expenditure (REE) in Amyotrophic lateral sclerosis (ALS) is the reflection of a high energy metabolic level, but these alterations seem controversial. Hypermetabolism (HM) is defined as a variation (_) > +10% between measured and calculated resting energy expenditure (REE). The main objective of the study was to confirm the existence of HM during ALS compared to healthy subjects. Secondary

objectives were to evaluate (i) if REE was increased in ALS patients without HM, and (ii) what was the level of REE.
Methods: A cohort of ALS patients was compared to a group of controls without metabolic disorders. The evaluation included anthropometric criteria measurements, REE by indirect calorimetry and body composition by impedancemetry (fat-free mass [FFM], fat mass). Statistical analysis used Mann-Withney and Chi2 tests. Multivariate analysis included logistic regression (main objective), and generalized linear models (secondary objectives).
Results: 315 patients and 80 controls were included. 55.2% of ALS patients had HM vs. 13.7% of controls (p <0.0001). HM was strongly and positively associated with the presence of ALS (OR= 9.50 [4.49 –20.10], p <0.0001). The metabolic level was higher in ALS patients (1503 kcal/24h [1290–1696] vs. 1220 kcal/24h [938 –1450], p <0.0001), even in the absence of HM (1339 kcal/24h [1154 –1543] vs. 1200 kcal/24h [1005 –1461], p <0.0001). The percentage of REE variation (_REE) and the REE/FFM ratio were higher in patients (p <0.0001). In multivariate analysis, _REE and the REE/FFM ratio were influenced by the group: ALS patients had a higher _REE compared to controls (_adjusted = 13.66% [10.31 –17.00], p < 0.0001) and REE/FFM ratio was higher during ALS (p < 0.0001), all the more than subjects were young.
Conclusion: This work confirms a metabolic deterioration and the increase of REE during ALS. This alteration could be considered as a marker of the disease. The identification of the mechanisms involved could improve patient management.

2071: **Predictive factors at time of diagnosis for gastrostomy and impact on survival in patients with amyotrophic lateral sclerosis**.

Marion Vergonjeanne (1,2)*, Philippe Fayemendy (1,2,3), Benoit Marin (1,2,4), Marie Penoty (1,2,5), Géraldine Lautrette (5), Huguette Sourisseau (3), Pierre Marie Preux (1,2,4), Jean Claude Desport (1,2,3), Philippe Couratier (1,2,5), Pierre Jésus (1,2,3).
1 INSERM, U1094, Tropical Neuroepidemiology, School of Medicine, Limoges, France.
2 Univ. Limoges, UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology, France
3 Nutrition Unit, University Hospital of Limoges, France
4 Epidemiology, Biostatistics and Methodological research centre, University Hospital of Limoges, France
5 ALS center, Neurology department, University Hospital of Limoges, France.

Background: A gastrostomy is recommended in ALS patients when the weight loss is over 10% as compared to usual weight, repeated aspirations, a meal time duration more than 45 minutes. The impact of gastrostomy on survival of ALS patients is still debated.
Objectives: i) to search at diagnosis, factors associated with the indication of gastrostomy ii) to evaluate survival of ALS patients with gastrostomy indication's according to their acceptance of the feeding tube placement.
Design: ALS patients were followed in the referral ALS centre between 2006 and 2017 and had from diagnosis to death prospective evaluations of their neurological, nutritional and respiratory status. Statistical analysis was done by using Mann-Whitney test, Chi_ tests, Cox model and multivariate logistic regression.
Results: Two hundred and eighty-five patients were included. Among the 63.9% for whom gastrostomy was indicated, 63.7% had accepted the placement. The median delays diagnosis-indication and indication-placement were 7.3 months [3.2 – 15.0] and 2.7 months [0.9 – 5.8], respectively. At diagnosis, bulbar onset, a loss of one point of body mass index and of bulbar functional scale were positively associated with indication of gastrostomy (aOR = 10.0; p = 0.002, aOR = 1.17; p = 0.025 and aOR = 1.19; p = 0.002, respectively). Weight loss > 5% significantly increased the risk of death by 17% (p < 0.0001). However, gastrostomy placement did not have impact on the survival (aHR = 1.25; p = 0.22).

Conclusion: Neurological and nutritional criteria were associated with an indication of gastrostomy. The gastrostomy placement had no impact on survival. The study of an earlier placement of gastrostomy and on the impact of the level of enteral nutrition associated might be of interest in further prospective studies.

2092: **Disease management analysis of new model of multidisciplinary care and its effects on survival in ALS patients.**

Riccardo Bonazzi (1), Andrea Lizio (2), Valeria Sansone (2,3), Christian Lunetta (2) .
1 Vivisol Srl, Monza, Italy.
2 Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milan, Italy.
3 Department of Biomedical Sciences of Health, University of Milan, Milan, Italy.

Disease management (DM) is defined as any medical or pharmaceutical intervention designed to improve both outcomes for the patient and overall cost-effectiveness of the health plan. ALS is a suitable condition for DM and can be used as a test case to generate short-term evaluable results. NEuroMuscular Omnicentre (Nemo) is a multidisciplinary hospital unit exclusively devoted to care and research of neuromuscular disorders, including ALS. During the 10 years of its activity, different structural and organizational changes were carried on to improve the quality of care. The aim of this study is to analyze the effect of the organizational clinical-assistance model of Nemo, identifying specific macro-stages, on survival of ALS.
ALS patients (pALS) followed in the NEMO Center from January 2008 to December 2018 were included. We identified the following stages: the run-in stage (2008-2009) based on an in- and out-patients multidisciplinary services; the consolidating stage (2010-2012), with development of an in-patients area for management of emergencies in ALS; the clinical trial stage (2013-2014) with inclusion of devoted research staff for clinical trials; the nurse-coach stage (2014-2016) with inclusion of a professional that integrated clinical, coaching and case management knowledge and direct interaction with homecare services; the Clinical Research Center stage (2017-2018), with development of a new area specifically devoted to out-patient service and clinical trials.
A total of 1212 pALS were recruited. Survival analysis showed a trend of improvement in patients that started the care in NEMO in the II, III and IV stage, reporting respectively a mortality rate of 20% (HR: 1.193), 25% (HR: 1.249) and 14% (HR: 1.141) lower than pALS started the care in the I stage, although with no significant p-values. However, pALS that started the care in the V stage reported a significant better survival compared to the patients belonging to the IV (HR: 1.70; p=.0011), III (HR: 1.53; p=.0079), II (HR: 1.57; p=.0022) and I stage (HR: 1.88; p<.0001).
This analysis confirmed that ALS is a good model to evaluate in a relatively short-time of assessment. Moreover, the stages of development of NEMO, the first hospital unit exclusively devoted to neuromuscular disease, showed a significant effect on ALS survival underling the importance, in the absence of a pharmacological therapy more active to influence survival, to develop model of ALS multidisciplinary care

2093: **The clinical value of motor nerve biopsy in Lower Motor Neuron Syndromes.**

Nilo Riva MD PhD (1,2),* Francesco Gentile MD (1), Federica Cerri MD PhD (1,2), Francesca Gallia MD (3), Yuri Matteo Falzone MD (2), Giancarlo Logroscino MD (4), Andrea Calvo MD (5), Giuseppe Lauria MD (6), Alberto Lazzerini MD (6), Massimo Corbo MD (7), Sandro Iannaccone MD (8), Christian Lunetta MD (9), Adriano Chiò MD (3) Eduardo Nobile-Orazio MD (6), Angelo Quattrini MD (1).

1) Experimental Neuropathology Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy
2) Department of Neurology, San Raffaele Scientific Institute, Milan, Italy
3) Neuromuscular and Neuroimmunology Service, Department of Medical Biotechnology and Translational Medicine, IRCCS Istituto Clinico Humanitas, Milano University, Milan, Italy.
4) Department of Neuroscience, University of Bari, Bari, Italy
5) Rita Levi Montalcini Department of Neuroscience, University of Turin, Turin, Italy
6) 3rd Neurology Unit and Motor Neuron Disease Centre, IRCCS Foundation Carlo Besta Neurological Institute, Milan, Italy and Department of Biomedical and Clinical and Sciences "Luigi Sacco", University of Milan, Milan, Italy
7) Department of Neurorehabilitation Sciences, Casa Cura Policlinico, Milan, Italy.
8) Department of Clinical Neurosciences, San Raffaele Scientific Institute, Milan, Italy.
9) Omnicentre (NEMO), Niguarda Ca Granda Hospital, Milan, Italy.

Introduction: We have previously shown that motor nerve biopsy may be used for an early diagnosis in lower motor neuron syndromes (LMNS). Herein, we defined the diagnostic performance of motor neve biopsy and 2015 new-revised El Escorial Criteria (NR-EEC) in amyotrophic lateral sclerosis (ALS) and ALS-mimics.
Methods: We retrospectively evaluated 90 LMNS patients with a diagnostic indication of motor nerve biopsy. Histopathologic and NR-EEC criteria were applied, and results compared to final diagnosis to assess their diagnostic utility in terms of sensitivity and specificity. Pathologic diagnosis was used in order to retrospectively validate the NR-EEC. Prognostic and therapeutic implications were further evaluated. We further explored whether TAR DNA-binding protein-43 (TDP-43) deposits in motor nerves might represent a useful biomarker for ALS.
Results: forty-nine were classified as MND (57%), seventeen as MN (19.8%), twenty as non-diagnostic (23.2%), while four were technically non-evaluable (4.4%). At follow-up, the pathological diagnosis showed a sensitivity of 78% for ALS and 85% for MN, with a specificity of 88.9% and 100%, respectively. NR-EEC showed a significant increase in sensitivity (45,9%) compared to R-EEC (11%). The degree of axonal degeneration was associated with shortened survival in ALS. A therapeutic response was observed in 66.7% of biopsy-proven-MN. TDP-43-immunoreactivity in motor nerves correlated with risk of developing ALS.
Conclusion: Motor nerve biopsy was conclusive in 76.4% of cases, correctly classifying 95.4% of pathologic biopsies as either ALS or MN. Expression of TDP-43 in motor nerve could be a potential biomarker for ALS.

1911: **Baseline audit of alternative and augmentative communication aid use by people with motor neurone disease in Scotland.**

Elizabeth Elliott*(2,3,4,6), Judith Newton (1,2,3,4,6), Phillipa Rewaj(2,3), Jenna M Gregory(2,4,6), Lynda Tomarelli(2,3), Shuna Colville(1,2,3,4,6), Siddharthan Chandran(1,2,3,4,6), Suvankar Pal(1-6) on behalf of the CARE-MND Consortium(1)
1 Clinical Audit Research and Evaluation for Motor Neurone Disease, Scotland, UK.
2 Euan MacDonald Centre for Motor Neurone Disease Research, University of Edinburgh, UK.
3 Anne Rowling Regenerative Neurology Clinic, Royal Infirmary, Edinburgh, UK.
4 NHS Lothian, UK.
5 NHS Forth Valley, UK.
6 Centre for Clinical Brain Sciences, University of Edinburgh.

Objectives: People with motor neurone disease (pwMND) experience speech dysfunction which can be supported by alternative and augmentative communication (AAC). We conducted a baseline audit of communication support for pwMND in Scotland against NICE guidance to inform and improve future service provision.

Methods: A cross sectional population based audit was undertaken. Anonymised demographic and clinical phenotypic data for all pwMND in Scotland were extracted from the Care Audit Research Evaluation of MND (CARE-MND) platform, the National MND Register for Scotland. Additional information for AAC provision was provided by the third sector charitable organisation MND Scotland (MNDS).
Results: 371 pwMND were included in the analysis, 43% of all pwMND were recorded as having impaired speech (recent ALSFRS-R score assessment ≤3) and 69% of all pwMND had been referred to speech and language therapy (SALT) services although there was significant variation in referral time.
36% of all pwMND were using a range of mainly high technology AAC to support either speech and/or limb dysfunction. The most frequently AAC used included; iPads, eye tracking technology and the LightwriterTM speech generating device.
Conclusions: Over a third of all pwMND across a range of MND disease subtypes were using AAC equipment to support speech and/or limb dysfunction. Early access to SALT services is advised to enable prospective and personalised decision making. Further qualitative research is required to understand the preferences and impact of AAC from the perspectives of the user and their communication partners.

1992: **Shape and size of Zagreb ENCALS centre ALS population.**

Barbara Sitas* (1), Hrvoje Bilic (1), Mirea Hancevic (1), Branimir Ivan _epec (1), Davorka Vranje_ (1), Gordana Pavli_a (2), Nedjeljka Zrno (3), Snjezana Svedi (1), Ervina Bilic (1).
(1) Neurology Department, Division for neuromuscular diseases, University Hospital Centre Zagreb, Croatia.
(2) Pulmology department, Division for respiratory insufficiency and pulmonary circulation diseases, University Hospital Centre Zagreb, Croatia.
(3) Pediatric Neurology Department, University Clinical Hospital Mostar, Bosnia and Herzegovina.

Becoming a part of ENCALS network we started our own ALS patients' registry. Here we present some of our epidemiological data. We retrospectively analysed medical history data for ALS patients' seen in Referral Centre for neuromuscular disorders, Department of Neurology; University Hospital Centre Zagreb (Centre). These patients have been diagnosed with ALS over the course of last three years.
Our database currently has 50 patients. There are 33 male and 17 female patients with a 1,9:1 gender ratio. Median age of first symptom onset is 59.4 years with 57.8 for men and 62.6 for women. We observed spinal onset in 74% of our patients. There is a difference in median age of onset depending on the site of onset - 56.9 for the spinal onset group and 67.6 for the bulbar onset group. We found no significant gender difference when it comes to spinal or bulbar onset Time from symptom onset to diagnosis was 13.7 months but after dividing patients into spinal and bulbar onset group it became clear that bulbar onset patients were seen and diagnosed faster with time to diagnosis 11.2 months compared to 15.3 months for spinal onset. Four of our patients have a sibling with ALS.
Patient number in our registry is slightly higher than what would be expected from regional ALS annual incidence for our population (800 000). Relatively high number of patients in the database is probably due to the fact our Centre is a National Referral Centre for neuromuscular disorders and receives countrywide referrals. This can also explain diagnostic delay that is evident in some cases as well as insufficient follow-up frequency which limits detailed disease progression monitoring. Shorter time to diagnosis for bulbar onset patients is probably due to higher symptom visibility leading to prompter referral. Median age in our database is comparable to previous epidemiological reports.
Although incomplete and still emerging we are pleased to see that our basic epidemiological data is in correlation with literature. We hope that with our "ALS network" we will provide optimal multidisciplinary treatment to patients countrywide regardless of their place of residence. This registry will also serve as an auditing tool which will help us improve care

but also as an educational tool for spreading awareness about importance of timely diagnosis and multidisciplinary care in ALS.

2063: **Subcutaneous glycopyrrolate reduces the amount of saliva in patients with motor neuron disease.**

Eva Žitnik(1*), Luka Rizmal(2), Blaž Koritnik (1), Leja Dolenc Grošelj (1), Janez Zidar (1), Lea Leonardis(1).
1 Institute of Clinical Neurophysiology, University Medical Center Ljubljana, Ljubljana, Slovenia.
2 Department of Neurology, University Medical Center Ljubljana, Ljubljana, Slovenia.

Objectives: Motor neuron disease (MND) is a progressive neurodegenerative disease involving upper and/or lower motor neuron degeneration. Although patients with MND produce normal amounts of saliva, swallowing problems due to bulbar dysfunction may cause sialorrhea. Many guidelines and recommendations for management of sialorrhea are established but only limited quantitative evidence support their efficacy. Glycopyrrolate (GLYC) is one of the most frequently used first-line anticholinergic medications for excessive salivation in patients with MND. In our study we were interested in the extent of saliva reduction after application of GLYC.
Materials and methods: Patients with MND and sialorrhea were invited to participate in our study. A pre-weighed cotton roll was placed sublingually for a period of five minutes and after that the mass of a wet cotton roll was measured. Subsequently patients received 100 mcg of GLYC subcutaneously. Ninety minutes after GLYC administration a new pre-weighed cotton roll was placed sublingually for a period of five minutes and the mass of a wet cotton roll was measured again. Saliva mass was calculated by subtracting the mass of pre-weighted cotton rolls from the mass of wet cotton rolls. The amounts of saliva produced before and after GLYC administration were compared.
Results: Ten patients (eight men) were included. Ninety minutes after subcutaneous GLYC administration the amount of saliva reduced on average by 49,3 ±14,7%.
Conclusion: GLYC subcutaneously significantly reduces the amount of saliva in patients with MND. It is a potential drug for reduction of sialorrhea.

References: 1. McGeachan A. J. et al. A multicentre evaluation of oropharyngeal secretion management practices in amyotrophic lateral sclerosis, Amyotroph Lateral Scler Frontotemporal Degener. 2017 Feb;18(1-2):1-9./2. Young CA, Ellis C, Johnson J, Sathasivam S, Pih N. Treatment for sialorrhoea (excessive saliva) in people with motor neuron disease/amyotrophic lateral sclerosis. Cochrane Database Syst Rev 2011(5):CD006981. Available at http:// onlinelibrary.wiley.com Acessed April 10, 2014.

MODELS:

1906: **Blocking or downregulation of Carnitine palmitoyl-transferase 1 (CPT1) delays disease progression in the SOD1 G93A mouse model.**

Michael Sloth Trabjerg (1*), Dennis Christian Andersen (1), Pam Huntjens (1), Ivo Huijbers (2), Colin Pritchard (2), Lona Kroese (2), John Dirk Nieland (1).
1. Laboratory of Metabolism Modifying Medicine, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark.
2. MCCA transgenic facility, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Amyotrophic lateral sclerosis (ALS) has long been recognized as a motor neuron disease. However, several recent findings indicates that ALS affects several systems outside the central nervous system. One key aspect in ALS is dysregulation of metabolism in general and alteration of lipid metabolism in particular. A key molecule in lipid metabolism is CPT1 and several studies indicate that CPT1 is upregulated in ALS. Preliminary data indicated that blocking CPT1 from day 100 in a SOD1 G93A mouse model was able to delay disease progression. Therefore, the aim of this study was to examine the effect of blocking CPT1 in the SOD1 G93A animal model of ALS from day 70 and genetic downregulation of CPT1A respectively by crossing SOD1 G93A mice with cpt1a p479l mice.
Male B6.Cg-Tg(SOD1*G93A)1Gur/J mice were bought from Jackson Laboratories and mated with female C57Bl6/J. Female transgenic mice and their wildtype littermates were randomized into treatment with the CPT1-blocker, etomoxir, or placebo from day 70. Mice were weighed and assessed weekly with a neurological score system, grip strength test, hangwire test and rotarod test from day 70 until they reached 150 days. Male B6.Cg-Tg(SOD1*G93A)1Gur/J mice were crossed with B6J-Cpt1a mice to generate a SOD1 G93A mouse with knockdown of CPT1A. These mice we evaluated by weight, neurological score, grip strength and hangwire test from day 70. B6J-Cpt1a mice were generated in collaboration with Netherlands Cancer Institute.
SOD1 G93A mice treated with the CPT1-blocker had significant later disease onset, significant lower disease score, improved grip strength and performed significant better at the hangwire test compared to the placebo group. Results from the SOD1 G93A mice with genetically knockdown of CPT1A are still pending but will be presented at the conference.
The data obtained in this study indicates that blocking lipid metabolism through the blockade of CPT1 has the potential to be a highly effective treatment of ALS.

1908: **Superoxide dismutase catalyzes the oxidation of thiol compounds to produce hydrogen peroxide.**

Shamchal Bakavayev (1), Nimrod Chetrit (1), Tatiana Zvagelsky (2), Maria Vyazmensky (1), Zeev Barak (3), Adrian Israelson (4), and Stanislav Engel (1)*.
1 Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
2 Department of Chemistry, Faculty of Natural Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
3 Department of Life Sciences, Faculty of Natural Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
4 Department of Physiology and Cell Biology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

A significant fraction of familial (fALS) and probably sporadic (sALS) amyotrophic lateral sclerosis (ALS) cases has been attributed to the toxic gain-of-function of misfolded Cu/Zn-superoxide dismutase (SOD1) enzyme. The mechanism of SOD1-dependent neurotoxicity, however, remains obscure. Here, we demonstrated that metal-saturated SOD1WT (holo-

SOD1WT) is capable of catalyzing the -oxidation of thiol compounds, with cysteine as the primary substrate, to produce H2O2. Although the holo-SOD1WT fails to oxidize glutathione (GSH), the major cellular antioxidant, in the presence of small quantities of cysteine, GSH turns into a potent pro-oxidant that donates reducing equivalents to fuel sustained cysteine-dependent H2O2 production. We propose that under certain metabolic circumstances, the GSH/cysteine couple may constitute a redox short-circuit capable of discharging cellular antioxidant potential by draining its GSH stores. In addition, the described reaction qualifies biological thiols as potential reducing agents to activate structurally impaired (misfolded) SOD1 to generate especially toxic ROS, namely hydroxyl radical and peroxynitrite, against which no enzymatic defense exists. By analyzing the distribution of thiol compounds throughout the CNS, the location of potential hot-spots of ROS production can be deduced. These hot-spots may constitute the origin of oxidative damage to neurons in ALS.

1922: **Behavioural impairments in transgenic mice expressing C-terminally truncated human FUS.**

Lysikova(1)*, N.Ninkina(1),(2), V.L.Buchman(1),(2).
1) Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Russian Federation.
2) School of Biosciences, Cardiff University, Cardiff, United Kingdom.

Malfunction of DNA/RNA-binding protein FUS causes certain forms of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Between molecular mechanisms of pathology shared by these diseases, pathogenic aggregation of FUS plays a crucial role. The clinical patterns reflect the predominant localisation of the pathological process in the nervous system: ALS is characterised by the degeneration of motoneurons that causes severe movement disorder, whereas the major target of pathology that leads to cognitive dysfunction in FTLD patients are neurons of the frontal and temporal cortex.
In healthy neurons FUS is involved in various steps of RNA processing and transport and is mainly localised in the nucleus but also shuttles to the cytoplasm. Most common disease-associated mutations of the FUS gene affect the C-terminal nuclear localisation signal of the encoded protein causing its accumulation in the cytoplasm.
Previously we have described transgenic mouse line expressing high level of a C-terminally truncated human FUS protein (FUS 1-359), which caused FUSopathy with severe motor phenotype. Analysis of another line, L_FUS[1-359], revealed a similar number of tandemly arranged copies of the same expression cassette located at Chr 11 but the level of human FUS expression in the nervous system of these mice was substantially lower than in the first line with different genomic location of the cassette at Chr 12. Although RNA sequencing identified a set of genes that change their expression in the spinal cord of these mice. Immunohistochemistry with antibody specific for N-terminal fragment of human FUS revealed diffuse pattern of staining in the cytoplasm of neurons throughout the nervous system of of L_FUS[1-359] mice but most profound accumulation of the truncated human FUS was observed in neurons of frontal and temporal cortex. This might explain behavioural changes observed in these mice. Homozygous but not hemizygous for transgenic cassette 5-month old L_FUS[1-359] mice displayed decreased anxiety in the dark-light box and elevated O-maze tests, and their social interaction in a resident-intruder test was also substantially decreased when compared to WT control mice.
This study was supported by Russian Science Foundation project _ 18–15-00357. Bioresource Collection of IPAC RAS (No. 0090-2017-0016) facilities were used to maintain transgenic mice and test their behaviour using equipment of the Centre for Collective Use IPAC RAS.

1927: **Elucidating the role of mutant fused in sarcoma (FUS) oligodendrocytes in the pathophysiology of ALS using induced pluripotent stem cells (iPSCs).**

*Katrien Neyrinck (1), Juan Garcia-Leon (1), Kristel Eggermont (1), Wenting Guo (2), Ludo Van Den Bosch Ludo (2), Catherine Verfaillie (1)
(1) Stem Cell Institute Leuven, Department of Development and Regeneration, KU Leuven, Belgium.
(2) Laboratory for Neurobiology, Department of Neurosciences, KU Leuven, Belgium.

FUS-linked amyotrophic lateral sclerosis (ALS) is responsible for an aggressive form of the disease, characterized by a juvenile onset and short survival. FUS mutations are most commonly located at the R521 position of the protein, while the P525L mutation causes the most severe phenotype making both mutations of interest to investigate. Although motor neurons are the main target in ALS, there is mounting evidence that oligodendrocytes also play a role in the disease. Spinal cord oligodendrocytes of FUS mutant ALS patients show signs of degeneration and pathological cytoplasmic aggregates. To investigate the role of oligodendrocytes in FUS-linked ALS we here use iPSC-derived oligodendrocytes. Human FUS(R521H) and FUS(P525L) mutant iPSCs and their isogenic controls were differentiated towards O4+ early oligodendrocytes by lentiviral SOX10 overexpression. Interestingly, compared with normal donor iPSCs and the isogenic control iPSCs, FUS mutant iPSCs generated less O4+ cells which was more pronounced for the FUS(P525L) cells. In a first analysis, qRT-PCR was used to quantify levels of a limited number of transcripts of stress pathways as well as lipid metabolism to address candidate mechanisms that might cause this phenotype. We observed that mutant FUS iPSCs and their progeny, at multiple time points during differentiation, expressed lower transcript levels of XBP1 ($p<0.01$), ATP5A1 ($p<0.05$), HMGCR ($p<0.05$) and ELOVL7 ($p<0.05$) suggesting defects in endoplasmic reticulum stress, ATP production and/or lipid metabolism. To complement this initial data, whole genome RNA sequencing studies are ongoing. As toxicity due to lentiviral transduction might contribute to these phenotypes, we are also creating patient and isogeneic iPSCs that have the inducible SOX10 cassette integrated in the safe harbor locus AAVS1. In conclusion, differentiation of FUS mutant ALS iPSCs towards O4+ late oligodendrocyte precursors appears defective, although the mechanisms underlying this phenotype remains to be fully clarified.

1933: **A non-canonical senescence profile in the spinal cord of the ALS model hSOD1-G93A.**

Pascual Torres* (1), Carlos Anerillas (2), Mario Encinas (2), Mònica Povedano (3), Reinald Pamplona (1), Manuel Portero-Otín (1).
1. Metabolic Pathophysiology Research Group, Department of Experimental Medicine, University of Lleida-IRBLleida, Lleida, Spain.
2. Oncogenic signalling and development, Department of Experimental Medicine, University of Lleida-IRBLleida, Lleida, Spain.
3. ALS Unit, Bellvitge Hospital, L'Hospitalet de Llobregat, Spain.

Aging is a major risk factor for developing amyotrophic lateral sclerosis (ALS). The Senescence-Associated Secretory Phenotype (SASP) is one of the changes associated with aging. SASP is characterized by the induction of some pro-inflammatory cytokines (Il1a, Il6), an increase in the expression of cell cycle inhibitors (p16-INK4a) and a higher amount of senescence-associated beta-galactosidase activity (SA-beta-gal), related to an increase on lysosomal biogenesis. SASP has been demonstrated to be noxious in age-related pathologies and cancer. Independent groups demonstrated the beneficial effects of eliminating senescent cells in Alzheimer's and Parkinson's diseases suggesting SASP role in age-related neurodegenerative diseases, without studying ALS. To fill this gap, we evaluated the expression of SASP markers (mRNA levels of Il1a, Il6, p16-INK4a, Ifna, Ifnb) and SA-beta-

gal activity by X-Gal assay in lumbar spinal cord of hSOD1-G93A mice in C57BL/6 background at 90 (pre-symptomatic), 120 (symptomatic) and 150 (end-stage) days. Moreover, we also evaluated TDP-43 splicing (dys)function, as a recently characterized marker of TDP-43-related alterations, by measuring the expression of Adipor2 cryptic exon by RT-qPCR. Results showed a progressive and significant increase of Il1a and p16-INK4a expression during the disease, with an increase of Il6 at end-stage. X-Gal assay showed a marked progressive decrease of SA-beta-gal activity, starting at 90 days. Regarding TDP-43 splicing function, G93A mice exhibited higher levels of Adipor2 cryptic exon only at end-stage with a positive correlation with p16-INK4a mRNA amounts. In conclusion, the G93A mouse model exhibits in spinal cord a senescence-like phenotype with a non-canonical SASP (without an increase in SA-beta-gal activity) associated with a late TDP-43 loss of function.

1934: **In vivo multiplexed chemogenetics reveals causal links in neuro-glio-vascular interactions in presymptomatic mutant SOD1 mice.**

Najwa Ouali-Alami (1), Linyun Tang (1), Albert C Ludolph (1), *Francesco Roselli (1)
(1) Ulm University, Ulm, Germany.

Disruption of the Blood-Spinal Cord-Barrier (BSPB) has been repeatedly reported in autopsy human spinal cord from ALS patients and in the mutant SOD1 murine ALS model. Although it is generally thought to be detrimental, solid evidence from direct in vivo manipulation of the BSPB is lacking; moreover, the causal relationship between neuronal dysfunction, glial activation and vascular disruption remains largely speculative since in vivo manipulation of identified cellular subpopulations at specific timepoints of the disease progression is necessary to shed light on this topic. Here we have exploited multiplexed in vivo chemogenetics using the PSAM/PSEM system and the DREADD system, combined with genetic (Cre-dependent or promoter driven) targeting of neurons or astrocytes through in vivo AAV injection to elucidate if BSPB barrier originates as consequence of neuronal dysfunction and of specific astrocyte signaling and if correction of BSPB defects have significant impact on disease burden. We demonstrate that i) BSPB structural and functional impairment is shared by multiple ALS mouse models ii) in the presymptomatic mutant SOD1 model, chemogenetic enhancement of motoneuron firing restores BSPB integrity, whereas the opposite is true when motoneuron firing is suppressed iii) activation of Gi signaling in astrocytes restores BSPB integrity without modifying disease markers in MN, whereas activation of Gq signaling reduces both disease markers and BSPB disruption iv) simultaneous suppression of motoneuron firing and activation of Gi signaling in astrocytes dissociates disease burden in MN (worsened) from BSPB integrity (preserved) v) chronic activation of Gi signaling in astrocytes prevent the disruption of the BSPB; however, BSPB integrity does not affect disease markers in motoneurons early on but does reduces disease markers burden later on. Thus, we demonstrate that BSPB disruption is initiated by dysfunction of motoneurons and modulated by astrocytes, and BSPB dysfunction worsens motoneuron disease markers only at later stages.

1935: **Large-scale remodeling of the motor subnetwork in mutant SOD1 mice revealed by projection mapping employing new retrograde AAV9.**

Barbara Commisso (1), Lingjun Ding (1), Karl Varadi (2), Martin Gorges (1), David Bayer (1), Tobias M Boeckers (1), Albert C Ludolph (1), Jan Kassubek (1), Oliver J Müller (3), *Francesco Roselli (1).
1) Ulm University, Ulm, Germany.
2) University Hospital Heidelberg, Germany.
3) University of Kiel, Germany.

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive degeneration of motoneurons in the primary motor cortex (pMO) and in spinal cord. However, the pathogenic process involves multiple subnetworks in the brain and functional MRI studies demonstrate an increase in functional connectivity in areas connected to pMO despite the ongoing neurodegeneration. The extent and the structural basis of the motor subnetwork remodeling in experimentally tractable models remain unclear. We have developed a new retrograde AAV9 to quantitatively map the projections to pMO in the SOD1(G93A) ALS mouse model. We show an increase in the number of neurons projecting from somatosensory cortex to pMO at presymptomatic stages, followed by an increase in projections from thalamus, auditory cortex and contralateral MO (inputs from 20 other structures remains unchanged) as disease advances. Accumulation of misfolded SOD1 is not the driver of the projection remodeling but, nevertheless, neurons involved in the network projecting to pMO simultaneously displays structural abnormalities and reduced spine density over time. The stage- and structure-dependent remodeling of projection to pMO in ALS may provide insights into the hyperconnectivity observed in ALS patients.

1937: **Large-scale structural remodeling of cortico-hypothalamic subnetworks associated with hypermetabolism in mutant SOD1 ALS model.**

David Bayer (1), Stefano Antonucci (1), Albert C Ludolph (1), *Francesco Roselli (1).
1) Ulm University, Ulm, Germany.

Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of motoneurons (MN) in the primary motor cortex and spinal cord. Although ALS is originally defined as a MN disease only, current studies suggest that ALS shows more systemic characteristics, including strongly altered metabolism. Recent findings uncovered that this energy metabolism changes occur initially before severe MN loss can be recognized, whereas the basis of this progressing pathogenic processes remains unclear. Since overt neuropathology has not been found in hypothalamus, we have hypothesized that changes in large-scale and local connectivity may contribute to the hypothalamic dysfunction and in particular of the lateral hypothalamus, which is responsible for food intake and energy balance.

Mapping large scale projections to lateral hypothalamus (LH) in the SOD1(G93A) ALS mouse model was achieved by using novel retrograde AAV2. To analyze the huge number of inputs to LH, a machine learning algorithms was trained to specifically detect neurons in different structures of the brain. The trained algorithm is able to differentiate artefacts, like crossing dendrites or auto fluorescent cells, from real neurons, as well as overlapping neurons by their individual shape, size and brightness. For registration, another customized software approach was used to map the sectioned brain according to stereotactic coordinates to the Allen Brain Atlas. Thus, automated and unbiased parcellation of the brain was achieved.

We have revealed that at symptomatic stage (P90, after the onset of body weight loss) significant chances in input to lateral hypothalamus from the prelimbic area and the medial part of the orbital area (decreased input to LH in ALS) as well as from the infralimbic area and agranular insular area (increased input to LH). The remaining input structures (more than 30) did not differ significantly between SOD1 and WT. Furthermore, breakdown of intra-hypothalamic connectivity was identified (both between left and right hypothalamus and within ipsilateral structures). These changes were not evident in presymptomatic mice (P30). This structure dependent remodeling of projections to HY in ALS provides insights into the altered connectivity and thereby more understanding of the structural basis of altered energy metabolism in ALS patients.

1941: **Presymptomatic ALS mice exhibit reversible functional and structural disruptions of glutamatergic proprioceptive synapses onto motoneurons**.

Marcin Bączyk (1,a,=), Najwa Ouali Alami (2,3,=), Clémence Martinot (1), Barbara Commisso (2,3), Linyun Tang (2,3), David Bayer (2,4), Nicolas Delestrée (1,b), Marin Manuel (1), Francesco Roselli (2,3,5,6), Daniel Zytnicki (1,*).
1) Center for Neurophysics, Physiology and Pathology, Paris Descartes University, CNRS UMR 8119, Paris, France.
2) Dept. of Neurology, Ulm University, Ulm, Germany.
3) Institute of Anatomy and Cell biology, Ulm University, Ulm, Germany
4) CEMMA research training group, Ulm University, Ulm, Germany
5) DZNE Ulm University, Ulm, Germany
6) Neurozentrum, Ulm University, Ulm, Germany.
a) Present address: Pozna_ University of Physical Education, Department of Neurobiology, Poznan, Poland/b) Present address: Columbia University, Department of Pathology and Cell Biology, New York, USA
= These authors equally contributed to this work.

Glutamate excitotoxicity has long been hypothesized to play a key role in motoneuron degeneration in Amyotrophic Lateral Sclerosis (ALS). However, whether or not excitatory synaptic transmission to motoneurons is enhanced before their degeneration has never been tested. Here we reveal that the opposite mechanism actually occurs. Indeed, in vivo intracellular recordings in anaesthetized mice demonstrate that EPSPs elicited by stimulation of Ia proprioceptive afferents onto motoneurons are on average 30% smaller in presymptomatic SOD1 G93A mice compared to control SOD1 WT mice. Moreover, short term plasticity of proprioceptive synapses, investigated using a paired-pulse protocol, switches from facilitation to depression. We show that these functional impairments are matched by deep structural disruptions of postsynaptic receptors and scaffold proteins, with no reduction in overall synapse density. Most interestingly, pathological glutamatergic transmission at the Ia-motoneuron synapse is partially rescued either by acute in vivo intracellular injection of cAMP analog during the course of electrophysiological experiments, or by acute or chronic chemogenetic activation (using DREADDs) of the PKA pathway that enhances the synaptic insertion of glutamate receptors. These experiments confirm the post-synaptic nature of the impairment. Moreover, double PSAM/DREADD chemogenetics experiments show that the harmful impact of inhibitory PSAM (which acts by reducing motoneuron excitability) on disease markers (LC3A, misfSOD1 and P62 aggregates are all elevated) is compensated by a chronic DREADD induced-PKA activation. We thereby propose the novel concept that a disruption of excitatory synapses onto motoneurons is a critical step of the disease pathophysiology.
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1961: **Differential distribution profile and toxicity of TDP-43N259S compared to TDP-43WT after overexpression in HEK293T cells.**

Débora Lanznaster*(1), Cindy Maurel(1), Laurie Lajoie(2), Patrick Vourc'h (1,3), Christian Andres (1,3), Philippe Corcia (1,4), Hélène Blasco (1,3).
(1) UMR 1253, iBRAIN, Université de Tours, INSERM, Tours, France.
(2) EA 7501 GICC (Groupe Innovation et Ciblage Cellulaire), Université de Tours, Tours, France.
(3) CHU de Tours, Service de Biochimie et Biologie Moléculaire, Tours, France.

(4) CHU de Tours, Service de Neurologie, Tours, France.

ALS is characterized by the presence of cytoplasmic aggregates of TDP-43 protein in degenerated neurons and surrounding cells. Identified mutations in TDP-43 accounts for 3% of familial and 1.5% of sporadic cases of ALS. Several studies reported different properties of wild-type TDP-43 compared to mutant forms of TDP-43 protein, including increase in aggregation, decrease in nuclear-cytoplasmic shuttling among others. Recently, our team described the first mutation in the RNA-recognition motif 2 (RRM2) domain of the TDP-43 protein (p.N259S), in a patient with a rapid form of ALS (Maurel et al., 2017). In the present study, we evaluated the properties of TDP-43 protein (p.N259S) in terms of localization, aggregation, mitochondrial membrane potential and viability in vitro, which could help us to understand the toxicity induced by this mutation. We induced TDP-43 proteins expression in HEK293T cells transfected with plasmids encoding GFP-TDP-43WT and GFP-TDP-43N259S. Flow cytometry experiments showed that TDP-43WT and TDP-43N259S express and aggregate similarly (p=0.4, TDPWT x TDPN259S). Flow imaging experiments allowed us to determine TDP-43 localization in cells and reveals that TDP-43WT localizes more in the cytoplasm than TDP-43N259S (p10 spots). TDP-43WT and TDP-43N259S are present similarly in the nucleus, but TDP-43WT cells present larger aggregates in the mitochondria than TDP-43N259S. Regarding mitochondria fragmentation, both TDP-43WT and TDP-43N259S cells present a decrease in spot counts compared to non-transfected cells (cells with 1-5 spots). However, TDP-43WT overexpression decreases the mitochondrial membrane potential ($p<0.05$) and cell survival ($p<0.05$) in comparison with TDP-43N259S. Our results show that TDP-43WT localizes more in the mitochondria, decreases it membrane potential followed by a decrease in cell viability when compared to TDP-43N259S. Besides the inherent limitations of our overexpression model, we can suggest that the different localization of the proteins studies here have an influence in their different toxic effects in HEK293T cells.

Reference: Maurel et al. Mutation in the RRM2 domain of TDP-43 in Amyotrophic Lateral Sclerosis with rapid progression associated with ubiquitin positive aggregates in cultured motor neurons. ALS & FTD, 2017; 1-3.

1990: **Characterization of mice with heterozygous TBK1 deletion.**

Clara Bruno (1), Kirsten Sieverding (1), David Brenner (1) and Jochen H. Weishaupt (1).
(1). Neurology department, Universitätsklinikum Ulm, Germany

Haploinsufficiency of tumor necrosis factor (TNF) receptor associated factor NF-_B activator (TANK)-binding kinase 1 (TBK1) has recently been shown to cause ALS and frontotemporal dementia (FTD). TBK1 regulates autophagy by phosphorylation of the autophagosome adaptor proteins p62 and optineurin, increasing their affinity to LC3-II and polyubiquitinated protein aggregates. Moreover, TBK1 is a central regulator of inflammation.

The aim of this project is to investigate the cellular and behavioral phenotype of Tbk1+/- mice until high age.

We show an increased accumulation of p62 protein in Tbk1+/- cortical neurons compared to the control group, as an indication of impaired autophagy in Tbk1+/- neurons in vitro.

Moreover, we observed a hyperactivity during first exposition to cognitive tests, but no decrease in motor abilities in Tbk1+/- mice vs. control group, even at 22 months of age.

Immunohistochemical analysis of 22 months old mice showed no difference in spinal motor neurons count. p62 aggregates showed a tendency towards increase in aged Tbk1+/- mice.

An increase of inflammatory markers was seen in the grey matter, but not in the white matter of 22 months old mouse spinal cords, with no difference between control and Tbk1+/- mice.

Immune system related gene expression analysis of more than 800 genes confirmed the age-dependent neuro-immune system alteration, but without substantial genotype-dependent alterations.
Here, we show that TBK1 haploinsufficiency does not lead to clinical symptoms in mice even at high age. However, we observed a subclinical phenotype with regard to p62 accumulation and neuroinflammation. Moreover, we found a robust age-dependent effect on microglial activation already in wild-type mice.

1994: **Restoration of histone acetylation ameliorates disease and metabolic abnormalities in a mouse model of amyotrophic lateral sclerosis.**

Elisabeth Rossaert* (1,2), Eveliina Pollari (1,2), Tom Jaspers (1,2), Lawrence Van Helleputte (1,2), Matthew Jarpe (3), Philip Van Damme (1,2,4), Katrien De Bock (5), Matthieu Moisse (1,2) and Ludo Van Den Bosch (1,2).
1 KU Leuven – University of Leuven, Department of Neurosciences, Experimental Neurology, and Leuven Brain Institute (LBI), Leuven, Belgium.
2 VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium.
3 Regenacy Pharmaceuticals Inc., Waltham, MA, USA.
4 University Hospitals Leuven, Department of Neurology, Leuven, Belgium.
5 Laboratory of Exercise and Health, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland.

Dysregulation of epigenetic mechanisms is emerging as a central event in neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS). In many models of neurodegeneration, global histone acetylation is decreased in the affected neuronal tissues. Histone acetylation is controlled by the antagonistic actions of two protein families –the histone acetyltransferases (HATs) and the histone deacetylases (HDACs). Drugs inhibiting HDAC activity are already used in the clinic as anti-cancer agents. The aim of this study was to explore the therapeutic potential of HDAC inhibitors in the context of ALS. We discovered that transgenic FUS-ALS mice, which recapitulate many aspects of human ALS, showed reduced global histone acetylation and alterations in metabolic gene expression, resulting in a dysregulated metabolic homeostasis. Chronic treatment of FUS-ALS mice with ACY-738, a potent HDAC inhibitor that can cross the blood-brain barrier, ameliorated the motor phenotype and substantially extended the life span of the FUS-ALS mice. At the molecular level, ACY-738 restored global histone acetylation and metabolic gene expression, thereby re-establishing metabolite levels in the spinal cord. Taken together, our findings link epigenetic alterations to metabolic dysregulation in ALS pathology, and highlight HDAC inhibitors as a potential therapeutic strategy to treat this devastating disease.

1996: **Analysis of the therapeutic potential of different administration routes and frequencies of human mesenchymal stromal cells in SOD1G93A mice.**

Franziska Bursch (1,2), Klaus Jan Rath (1,4), Anastasia Sarikidi (1,4), Sebastian Böselt (1,4), Ekaterini Kefalakes (1,4), Alma Osmanovic (1,4), Nadine Thau_Habermann (1), Stephan Klöß (3,4), Ulrike Köhl (3,4), Susanne Petri (1,2,4).
1 Department of Neurology, Hannover Medical School, Hannover, Germany
2 Centre for Systems Neuroscience (ZSN), Hannover, Germany
3 GMP Development Unit, Institute of Cellular Therapeutics, Hannover Medical School,Hannover, Germany
4 Integrated Research and Treatment Center for Transplantation, Hannover Medical School, Hannover, Germany.

Background: Cellular therapy represents a novel option for the treatment of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS). Its major aim is the generation of a protective environment for degenerating motor neurons. Mesenchymal stromal cells secrete different growth factors and have antiapoptotic and immunomodulatory
properties. They can easily and safely be isolated from human bone marrow and are therefore considered promising therapeutic candidates.
Methods: In the present study, we compared intraventricular application of human mesenchymal stromal cells (hMSCs) versus single and repeated intraspinal injections in the mutant SOD1G93A transgenic ALS mouse model.
Results: We observed significant reduction of lifespan of animals treated
by intraventricular hMSC injection compared with the vehicle treated control group, accompanied by changes in weight, general condition, and behavioural assessments. A potential explanation for these rather surprising deleterious effects lies in increased microgliosis detected in the hMSC treated animals. Repeated intraspinal injection at two time points resulted in a slight but not significant increase in survival and significant improvement of motor performance although no hMSC_induced changes of motor neuron numbers, astrogliosis, and microgliosis were detected. Quantitative real time polymerase chain reaction showed reduced expression of endothelial growth factor in animals having received hMSCs twice compared with the vehicle treated
control group. hMSCs were detectable at the injection site at Day 20 after injection into the spinal cord but no longer at Day 70.
Conclusion: Intraspinal injection of hMSCs may be a more promising option for the treatment of ALS than intraventricular injection. Repeated injections might be necessary to obtain substantial therapeutic benefit.

2016: **Investigating cerebellar alterations in the TDP-43(Q331K) knock-in mouse.**

Camille Hunt(1)(*), Selina Lin(1), Matthew White(1), Luis Emiliano Pena Altamira(1), Jemeen Sreedharan(1).
1 Maurice Wohl Clinical Neuroscience Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

More than 10 years ago, mutations in the Tardbp gene, which encodes the TDP-43 protein, were identified as a cause of ALS (Sreedharan, 2008). The majority of ALS cases (97%) and approximately half of FTD cases (45%) demonstrate TDP-43 inclusions in post-mortem analyses (Neumann, 2006, Arai 2006, Ling, 2013). TDP-43 clearly has a central role in the pathogenesis of the ALS-FTD spectrum. We recently showed that the introduction of the conserved Q331K mutation into murine Tardbp resulted in an FTD-like phenotype caused by perturbed TDP-43 autoregulation, increased TDP-43 expression and splicing changes of transcripts targeted by TDP-43 (White, 2018). Of interest was the increased inclusion of exons 2 and 3 of the Mapt gene, which encodes tau, another dementia-associated protein. To further investigate the links between TDP-43 and tau in ALS-FTD we used in vivo brain MRI to identify regions of interest in 7-month old male mice. In comparison to wild-type mice, TDP-43(Q331K) mice exhibited reduced volume in multiple regions within the frontal lobe (Lin, Oxford ENCALS 2018). Unexpectedly, we also found significant volume changes in the brainstem and cerebellum.
The cerebellum had long been considered an unaffected brain region in ALS-FTD, but increasing evidence shows that cerebellar atrophy can occur in ALS-FTD and contributes to cognitive symptoms (Gellersen, 2017). In the present study, we investigate the nature of the Q331K-related cerebellar volume reduction and the roles of TDP-43 and N-terminally alternatively spliced tau isoforms in the neurodegeneration of MRI affected brain regions.

Preliminary immunostaining has indicated morphological alterations in the molecular/granular layers of cerebellar lobules. Interestingly, we find an increase in microglial activation in TDP-43(Q331K) mice. Additionally, we have confirmed that perturbed TDP-43 autoregulation and Mapt splicing alterations occur in the cerebellum, however, the pattern of tau isoform expression differs from that seen in the frontal cortex. Although no differences in localization of tau were previously observed in frontal cortices of TDP-43(Q331K) knock-in mice, we are conducting more in-depth investigations using isoform-specific antibodies to elucidate how altered levels/localisation of tau isoforms and microglial activation contribute to disease. These studies ultimately aim to unravel how cerebellar dysfunction contributes to cognitive syndromes associated with in ALS-FTD.

2019 **MSC-derived exosomes normalize the activated phenotype of late symptomatic SOD1G93A mouse-derived spinal cord astrocytes by miRNA shuttle.**

Francesca Provenzano* (1); Carola Torazza (1); Marco Milanese (1); Debora Giunti (2); Chiara Marini (2); Benedetta Parodi (2); Cesare Usai (3); Nicole Kerlero de Rosbo (2); Antonio Uccelli (2,4); Giambattista Bonanno (1,4)
(1) Department of Pharmacy, Pharmacology and Toxicology Unit and Center of Excellence for Biomedical Research, University of Genoa, Italy.
(2) Department of Neurosciences Ophthalmology and Genetics and Center of Excellence for Biomedical Research, University of Genoa, Italy.
(3) Institute of Biophysics, National Research Council, Genoa, Italy.
(4) IRCCS San Martino Polyclinic Hospital, Genoa, Italy.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons (MNs) but involving also non-neuronal cells. Degeneration of MNs has been linked to neuroinflammation, supported mostly by activated glial cells. We have previously shown that the intravenous administration of mesenchymal stem cells (MSCs) in SOD1G93A mice prolonged survival, ameliorated motor skills and reduced gliosis and inflammation in spinal cord. The beneficial effects were not associated with MSC differentiation, being possibly mediated through paracrine mechanisms. We hypothesized that MSC-derived exosomes and exosome-shuttled miRNAs could mediate these positive effects. To verify our hypothesis, we tested exosomes derived from INFα-activated MSCs on cultured astrocytes prepared from the spinal cord of 120 day-old late-symptomatic SOD1G93A mice. Vimentin and GFAP expression were increased in SOD1G93A astrocytes vs. age-matched WT astrocytes ($p<0.001$) and their expression was reduced by 40% and 80%, respectively ($p<0.001$), after exposure to exosomes. We then examined the inflammatory pattern of SOD1G93A astrocytes. IL-1β, TNF-α and IL-6 were more expressed ($p<0.001$) and more efficiently released ($p<0.01$) in SOD1G93A astrocytes and the exposure to exosomes resulted in a significant decrease of their overexpression, by about 65%, 80% and 60%, respectively ($p<0.001$), and of their release ($p<0.05$). Conversely, IL-10 was decreased in SOD1G93A astrocytes ($p<0.001$) and its expression was normalized after exposure to exosomes ($p<0.001$). Also, NLRP3 expression was increased in SOD1G93A astrocytes ($p<0.001$) and the increase was reversed (70%) by exosomes ($p<0.001$). We also studied the impact of the astrocyte exosome treatment on MN survival. The viability of MNs seeded on exosome-treated SOD1G93A astrocytes was increased when compared to co-cultures with untreated astrocytes ($p<0.01$ at days 8 and 14; $p<0.001$ at days 6, 10 and 12). Finally, we tested nine miRNAs, which have been found up-regulated in activated MSCs and present in exosomes, by transfecting SOD1G93A astrocytes with the specific synthetic mimics. Seven of these miRNAs significantly reduced GFAP, IL-1β and TNF-α expression. These results indicate that exosomes and exosome-shuttled miRNAs can reduce astrocyte reactivity and that this

effect has a positive impact on MN viability. The in-vitro exosome activity paves the way to translational preclinical in-vivo treatments in SOD1G93A mice.

2023: **Increased macrophage recruitment through CCL2 overexpression prevents skeletal muscle atrophy and motor neuron loss in a mouse model of ALS.**

Maria Chiara Trolese, Valentina Melfi, Caterina Bendotti, Giovanni Nardo*
Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Via La Masa, 19, 20156 Milan, Italy.

Increasing evidence suggests that the immune system plays a controversial role in the pathogenesis and progression of Amyotrophic Lateral Sclerosis (ALS) and this may explain the failure of non-targeted anti-immune therapies in clinical trials.
Several compelling investigations have established the importance of CCL2-mediated signaling in driving both axonal and muscle inflammation and regeneration following injury. We recently showed that SOD1G93A mice with slower disease progression (C57SOD1G93A) expressed higher levels of CCL2 in the motor neurons (MNs), axons and nerve terminals with respect to those with fast disease course (129SvSOD1G93A mice). This effect correlated with increased macrophage infiltration in sciatic nerves and hindlimb muscles while this response was less evident in rapidly progressing mSOD1 mice with higher skeletal muscle denervation atrophy.
To clearly demonstrate that MN-mediated CCL2 overexpression and muscle-macrophage interaction plays a critical role for the maintenance of neuromuscular function in ALS, we overexpressed the chemokine in myofibers and MNs through the injection of self-complementary (sc) AAV9-CCL2 in the skeletal muscles of adult mSOD1 mice.
The overexpression of CCL2 in C57SOD1G93A mice promoted the recruitment of hematogenous macrophages driving their conversion to an M2-phenotype in skeletal muscles. This was accompanied by protection of MNs in the lumbar spinal cord and a reduced muscle denervation atrophy with consequent amelioration of the motor performance and a delay in the disease onset of about three weeks.
On the contrary, a similar overexpression of CCL2 in the neuromuscular system of 129SvSOD1G93A mice failed in the recruitment of macrophages and had no effect on muscle atrophy. These results clearly indicate that the recruitment of macrophages mediated by CCL2 in the peripheral compartment of the neuromuscular system is essential to maintain a regenerative capacity of the motor neuron and muscle during ALS disease progression. Understanding the mechanism underlying this phenomenon and why is deficient in 129SvSOD1G93A mice, may open the way to identify novel targets to promote regeneration and useful index to stratify patients for the clinical trial.
The study was supported by the (TRANS-ALS) - Regione Lombardia (no. 2015–0023).

2024: **Evaluation of the effects of an agonist antibody of the FGF21 pathway in a mouse model of Amyotrophic Lateral Sclerosis (ALS).**

JB Delaye*(1), D Lanznaster(2), A Lefevre(2), P Vourc'h(1)(2), JC Lecron(3), CR Andres(1)(2), P Corcia(2), H Blasco (1)(2).
(1) Laboratoire de Biochimie et de Biologie Moléculaire, CHRU de Tours, 37044 Tours, France
(2) UMR U 1253, Equipe 2 Neurogénétique et Neurométabolomique, 37032 Tours, France.
(3) Laboratoire Inflammation, Tissus Epithéliaux et Cytokines EA 4331 Université de Poitiers, France.

Many pathophysiological ways have been identified in ALS and an interesting therapeutic approach could be the use of molecules targeting different mechanisms at the same time.
In this context, the Fibroblast growth factor 21 (FGF21) pathway, which is linked to inflammation and energy metabolism, could be a potential therapeutic target. Thus, we

evaluated the clinical behavioral and biological effects of a monoclonal antibody agonist of the FGF21 pathway (R1Mab1) in a mouse model of ALS.
Four groups (transgenic SOD1*G93A and wild type WT, treated intraperitoneally with R1Mab1 or drug vehicle (DV); n=12) were explored. The behavioral phenotype was evaluated by the following parameters: rotarod test, weight measurement, and muscle mass evaluation by high resolution ultrasound. The biological effects were evaluated by the exploration of hormones involved in energy metabolism (like insulin, leptin, resistin) and some mediators of the inflammation (TNF-α, IL-6, MCP-1, PAI-1). Serum metabolomic profile was also determined by mass spectrometry to have an overview of the metabolism without any a priori.
The R1Mab1-treated mice had a significant weight loss with a lower weight from the week 13 to 20 ($p < 0.001$). This weight loss was greater in the SOD1*G93A vs WT mice ($p < 0.01$). A limited effect on rotarod performance degradation occurred at week 20 in SOD1*G93A + R1Mab1 vs SOD1 * G93A + DV ($p = 0.032$). Ultrasound results showed a significant increase in the ratio of muscle area to weight in SOD1*G93A + R1Mab1 vs SOD1*G93A + DV mice ($p = 0.036$).
There is a decrease in pro-inflammatory cytokines (TNF-α and MCP-1) at week 16 in SOD1 * G93A + R1Mab1 vs SOD1 * G93A + DV (respectively $p = 0.0059$ and $p = 0.003$). The results of metabolomic analysis are still in progress and should provide a better understanding of the metabolic pathways involved.
At our knowledge, this study is the first one evaluating the FGF21 pathway in an ALS mice model. We showed the potential interest of this pathway with an improvement of the inflammatory state and a limited effect on the degradation of the motor performances despite the significant weight loss. We expect informative findings from metabolomics in order to better characterize the mechanism of action of R1Mab1 and also to describe the biological evolution related to ALS progression in this mouse model.

2031: **Comparison of iPSC-derived motor neurons from C9ORF72 patient fibroblasts and peripheral blood cells: a proof-of-principle study**.

Donatella Bardelli (1)(*), Francesca Sassone (1), Clara Volpe (2), Claudia Colombrita (1), Valentina Gumina (1), Silvia Peverelli (1), Antonia Ratti (1,2), Vincenzo Silani (1,3,4) and Patrizia Bossolasco (1).
1 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan and Cusano Milanino, Italy.
2 Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan 20122, Italy.
3 "Dino Ferrari" Centre, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.
4 "Aldo Ravelli" Center for Neurotechnology and Experimental Brain Therapeutics, Università degli Studi di Milano, Milano 20122, Italy.

Induced Pluripotent Stem Cells (iPSCs) are commonly obtained by reprogramming fibroblasts although many different cell types can be used, and are useful to deepen disease mechanisms and therapeutic strategies. In this study we decided to compare iPSCs and iPSC-derived motor neurons obtained from both fibroblasts and peripheral blood mononuclear cells (PBMCs) of the same ALS patient carrying a C9ORF72 expansion, in order to demonstrate the equivalence of the two starting sources. To obtain iPSCs, we used the CytoTune-iPS 2.0 Sendai Reprogramming Kit. An iPSC clone from both tissues was fully characterized for the expression of stemness markers by immunocytochemistry and RT-PCR, and we evaluated its ability to spontaneously differentiate into the three germ layer cells. We also verified the normal karyotype and the maintenance of the GGGGCC expansion after reprogramming by a repeat-primed PCR. The iPSCs were then

differentiated into motor neurons and the expression of Hb9 and SMI312 was evaluated by immunofluorescence, as well as the eventual cytoplasmic mislocalisation of TDP-43 and presence of aggregates. Furthermore, we analyzed in iPSC-derived motor neurons a specific hallmark of C9ORF72 mutated cells, consisting in the generation of sense and antisense RNA foci by FISH technique. We did not observe any difference in the reprogramming of fibroblasts or PBMCs neither in the iPSC differentiating ability. In addition, the number of foci observed in iPSCs and iPSC-derived motor neurons was comparable between the two starting tissues. Using Southern Blot analysis, we found a contraction of the hexanucleotide expansion in both iPSC clones.
In summary, this study pointed out that iPSCs and iPSCs-derived motor neurons from two different tissue sources, fibroblasts and PBMCs, display comparable in vitro features and behavior. Given the reduced ease of collecting skin biopsies and the better patient compliance in undergoing a blood sample collection, this proof-of-principle study could also have an impact in number of samples collected and possibility of bio banking.

2032: **A high throughput screen identifying modifiers of motor neuron survival and stress granule dynamics from a TDP-43 transgenic mouse model of ALS.**

David Gordon (1), Ruxandra Dafinca (1), Stephanie Hatch (2), Daniel Ebner (2), Kevin Talbot (1).
(1) Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK.
(2) Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK.

Background The mechanisms underlying the preferential loss of motor neurons (MNs) in ALS are still poorly understood, but may be due, in part, to a defective response to conditions such as oxidative stress. Mutations in many RNA binding proteins, such as TDP-43, the pathological hallmark protein of ALS, can impair the normal assembly of stress granules, suggesting a potential pathogenic mechanism. We have developed a transgenic mouse expressing human TDP-43 (M337V) at low levels, which develop distinct motor deficits (rotarod and grip strength), show survival deficits and pathological phenotypes (reduced axonal transport, NMJ deficits) recapitulating some key features of human ALS. In primary mouse spinal cord and embryonic stem cell (ESC)-derived MNs we used rescue of sodium arsenite-induced MN death and stress granule deficits as readout in automated screens to identify potential disease modifying drugs.
Methods Primary MNs were generated from E13.5 lumbar spinal cord from non-transgenic (NTg), TDP-43WT/- and TDP-43M337V/- embryos. Mouse ESCs (reflecting the same genotypes) were expanded as embryoid bodies and differentiated into MNs in 384-well plates, treated with 0.5mM sodium arsenite for 60 min then incubated with resazurin. Cell viability was assayed on an Incell 6000 confocal microscope. TDP-43M337V/- MN death was compared to stressed and unstressed controls.
Results TDP-43M337V/- primary MNs recapitulate TDP-43 cytoplasmic mislocalisation compared to NTg and TDP-43WT/- controls and was accompanied by altered stress granule dynamics. The presence of M337V TDP-43 in ESC-MNs results in a reduced proportion of ESC-MNs expressing stress granules, reduced stress granule size and less stress granules/ESC-MN. We also observed reduced stress recruitment of mutant TDP-43 to stress granules and significantly reduced survival in response to exposure to oxidative stress.
Conclusions Our data suggest that impaired stress responses may underlie the link between mutant TDP-43 and selective MN loss in our mouse model of M337V-associated ALS. With the Oxford Target Discovery Institute we are performing a screen of FDA approved compounds to identify drugs which might restore survival and the stress granule response in mutant-expressing ESC-MNs. Compounds will be validated through detailed analysis of

phenotypic and transcriptional changes in primary mouse MNs, and iPSC-derived motor and cortical neurons from ALS patients carrying TDP-43 mutations.

2042: **Genetic ablation of SOD1G37R selectively from corticospinal neurons improves their survival without affecting ALS onset and progression**.

Scekic-Zahirovic Jelena (1*), Fischer Mathieu (1), Stuart Lopez Geoffrey(1), Rouaux Caroline (1).
1. Inserm U1118, Université de Strasbourg, Faculté de Médecine, Strasbourg, France.

While evidence of combined degeneration of the bulbar and spinal motor neurons (MN/lower motor neurons) together with the corticospinal neurons (CSN/upper motor neurons) is required to diagnose Amyotrophic Lateral Sclerosis (ALS), preclinical studies have mostly concentrated on the MN, leaving aside the CSN and their contribution to ALS onset and progression. Selective removal of mutant SOD1G37R gene from MN slows early disease progression, while SOD1G37R excision selectively from the microglia slows late disease progression indicating the co-occurrence of cell-autonomous and non-cell autonomous effects on MN survival. Whether such cell-intrinsic and extrinsic effects on CSN survival exist, and what are their repercussions on disease onset and progression is unknown.
To address this question, we generated a mouse model overexpressing the mutant SOD1G37R transgene ubiquitously except in the CSN and other related sub-cerebral projection neurons of the cortical layer V. These mice were obtained by crossing the FloxedSOD1G37R mice to the Crym-CreERT2 mice that we recently developed, and that restricts Cre expression to the cortical layer V subcerbral projection neurons that include CSN.
Using retrograde labelling, we show that genetic ablation of SOD1G37R selectively from CSN improves their survival, suggesting that CSN degeneration may rely, at least partly, on cell-autonomous mechanisms. In accordance with this, we detected reduced spasticity, a classical sign of CSN degeneration, in Crym-CreERT2; FloxedSOD1G37R mice compared to their FloxedSOD1G37R littermates. Finally, we tested whether CSN rescue could be beneficial for motor functions and survival. During the early phase of the disease, we observed that CSN-selective deletion of SOD1G37R ameliorated some motor performances. Yet disease onset and survival remained unchanged.
Altogether, the data indicate that CSN degeneration is, at least partly, cell-autonomous and, as expected, is correlated spasticity. Yet, maintenance of CSN is not sufficient to positively impact ALS onset and progression.

2048: **Partial genetic deficiency of granzyme A prolongs survival in an ALS mouse model.**

Laura Moreno-Martínez (1), Ana Cristina Calvo (1*), Llipsy Santiago (2), Janne M. Toivonen (1), Miriam de la Torre (1), Leticia Moreno (1), Nora Molina (1), Raquel Manzano (1), María Jesús Muñoz (1), Pilar Zaragoza (1), Julián Pardo (2,3), Rosario Osta (1).
(1) LAGENBIO (Laboratory of Genetics and Biochemistry), Faculty of Veterinary-IIS Aragón, IA2-CITA, CIBERNED-612, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain.
(2) Department of Biochemistry and Molecular and Cell Biology, and Department of Microbiology, Preventive Medicine, and Public Health, University of Zaragoza-IIS Aragón, 50009 Zaragoza, Spain.
(3) Aragon I+D Foundation (ARAID), Zaragoza, Spain.

The immune system is dysregulated in amyotrophic lateral sclerosis (ALS), contributing to exacerbate the course of the disease. Granzyme A (gzmA) is a serine protease involved in the modulation of the inflammatory immune response that has been found elevated in serum from ALS patients. However, it is not clear yet whether gzmA could influence on

progression of ALS disease. Thus, the aim of this work was to assess whether the absence of gzmA in an ALS murine model could help to slow down the progression of the disease. Homozygous and hemizygous gzmA deficient mice expressing the SOD1G93A transgene were generated by crossing the SOD1G93A transgenic and gzmA deficient mice. Survival of mice presenting the three gzmA genotypes was monitored and Gzma gene expression was measured in spinal cord and quadriceps from these mice. We observed the greatest lifespan in gzmA+/- mice, which was similar to the one observed in gzmA-/- mice, whereas gzmA+/+ showed the shortest survival rate. Gzma gene expression was downregulated in spinal cord from gmzA+/- mice, confirming that increased survival of hemizygous mice correlated with lower gzmA gene expression levels. In addition, mRNA levels of IL-1β a pro-inflammatory cytokine, and GSR, involved in oxidative stress, were also found downregulated in spinal cord from gmzA+/- mice. In summary, our findings indicate for the first time that reduced levels, but not the absence, of gzmA could ameliorate the disease progression in this animal model.

2059: **Investigating cell autonomous and non-cell autonomous effects in a TDP-43M337V BAC mouse model of Amyotrophic Lateral Sclerosis.**

Jayden Clark*, Myrna Kelfkens, David Gordon, Kevin Talbot.
Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom.

Background: A growing body of research has implicated non-neuronal cells in the onset and progression of MND/ALS. Activation of microglia and astrocytes occurs in the cortex and the spinal cord of ALS patients and rodent models, with microglia implicated in the progression and severity of the disease. However, it remains unclear whether microglia are a primary driver of ALS pathology, such as the neuropathological hallmark of TDP-43 mislocalisation from the nucleus to the cytoplasm in motor neurons. In this study we utilised a BAC-TDP-43 mouse model to investigate neuron-microglial interactions. Methods: Non-transgenic and TDP-43M337V mice were used to generate primary motor neuron and glial cultures. Motor neurons were also obtained from the differentiation of mouse embryonic stem cells (mESCs). Microglia were isolated and plated onto 1-day old motor neuron cultures and cultured for 48 hours. mESCs were treated with 20ng/ml TNFα. Microglial were activated using lipopolysaccharide (LPS), interferon gamma (IFNγ) and interleukin-4 (IL-4). Confocal microscopy, qRTPCR, western blot and ELISA were used to evaluate the motor neuron-microglial interaction and inflammasome pathways. Results: Treatment with LPS, or combined LPS/IFNy (proinflammatory) or IL-4 (anti-inflammatory) increased the respective activation state of non-transgenic and transgenic microglia. TDP-43M337V microglia adopt a proinflammatory phenotype, and show increased sensitivity to proinflammatory activators, compared to non-transgenic microglia. Proinflammatory activation causes a significant increase in TDP-43 expression in TDP-43M337V microglia, as well as an increase in cytoplasmic TDP-43 immunostaining. Mouse ESCs showed no difference in TDP-43 localisation when treated with TNFα. However, phosphorylation of NF-κB p65 is significantly increased in untreated TDP-43M337V mESCs, and is not further responsive to TNFa treatment. Motor neuron-microglial co-cultures suggest that TDP-43M337V microglia do not induce TDP-43 mislocalisation in primary motor neurons, or facilitate motor neuron degeneration in vitro. Discussion: This study investigates the non-cell autonomous pathophysiological mechanisms of microglia in TDP-43 mislocalisation and motor neuron survival. Preliminary results suggest that TDP-43M337V mutant microglia show a robust proinflammatory profile. However, inflammatory cytokines and direct cell-cell interactions may not drive the mislocalisation of TDP-43 in motor neurons.

2062: **Selective and presymptomatic corticospinal neuron degeneration is associated with developmental RNA dysregulation in a SOD1 model of ALS.**

Christine Marques (1), Mathieu Fischer (1), Jelena Scekic-Zahirovic (1), Thibaut Burg (1), Aurore Brunet (1), Céline Keime (2) and Caroline Rouaux*(1).
1 Inserm UMR_S 1118, University of Strasbourg; Faculté de Médecine; Strasbourg, France.
2 Inserm UMR_S 964, CNRS UMR_S 7104, Université de Starsbourg, IGBMC; Strasbourg, France.

Amyotrophic lateral sclerosis (ALS) is clinically defined as the combined degeneration of the corticospinal and corticobulbar motor neurons (CSN) along with the bulbar and spinal motor neurons (SMN). While a growing body of evidence points to the cerebral cortex has the potential initiation site of ALS, little is known about the spatiotemporal dynamics of CSN degeneration, and the molecular pathways involved. Here, we used retrograde labelling in combination with rigorous sampling and counting of either the whole CSN population or only the lumbar-projecting CSN, and showed that in Sod1G86R CSN loss occurs in a somatotopic manner and precedes weight loss, motor symptom appearance and SMN degeneration. To gain insights into the molecular mechanisms that selectively trigger CSN degeneration, we purified adult disease-sensible CSN and disease-resistant callosal projection neurons (CPN) from the cerebral cortex of wild-type or Sod1G86R mice at different time points of the disease, and conducted a longitudinal of RNAseq investigation. Comprehensive analysis of the data indicates that CSN dysfunction and degeneration is accompanied, among other dysregulations, with altered RNA metabolism. Analysis of the splicing events further confirmed altered RNA metabolism in the CSN of the Sod1G86R mice, and identified common missplicing events across different mouse models of ALS. Finally, analysis of the embryonic cortex Sod1G86R mice suggest that some of the transcriptionnal and splicing alterations seen in adult CSN may arise as soon as they generated.

2074 : **Characterization of a novel FUS zebrafish model to study ALS**.

Annis-Rayan Bourefis*(1)(2), Maria-Letizia Campanari(1)(2), Edor Kabashi(1)(2).
(1) Sorbonne Université, Université Pierre et Marie Curie (UPMC), Université de Paris 06, Unité Mixte 75, Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1127, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche 7225 Institut du Cerveau et de la Moelle Épinière (ICM), 75013, Paris, France.
(2) Imagine Institute, INSERM Unité 1163, Paris Descartes Université, 75015, Paris, France.

FUS is an RNA-binding protein involved in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Altered FUS nuclear localization and cytoplasmic FUS aggregates are found in ALS patients carrying FUS mutations, as well as in a subset of FTD patients. Since the discovery of the gene, a number of animal models have been developed to study its mechanisms but, among all these studies, FUS-dependent mechanisms in ALS remain unclear. Thus, further models are needed to better understand the role of this factor in ALS pathogenesis. The objective of this work here is to characterize a novel FUS zebrafish model to study pathogenic mechanisms leading to ALS and related disorders.
Here, we describe for the first time a genetic mutant model for FUS in zebrafish with relevant phenotypic features. In this genetic line, we observed in homozygous animals consistently reduced life span as well as a severe motor phenotype either in stimulus-induced locomotion or in spontaneous locomotion tests. These behavioral deficits were accompanied by anatomical defects, including reduced length of motor neurons, increased synaptic branches and neuromuscular junctions (NMJ) fragmentation. Moreover, the major motor defects were rescued by overexpression of human FUS mRNA but not by human TARDBP mRNA. Similarly, other factors functionally related to FUS, TAF15 were not altered showing that there was no major compensation by other RNA-binding proteins in the FUS

deletion mutant zebrafish. Finally, we show in this FUS zebrafish model a misregulation of MAPT paralogues, encoding for the protein Tau. Furthermore, the disregulation at the transcript level leads to increased levels of tau, a factor well-known to be implicated in FTD and Alzheimer. Similarly to other studies, this genetic model reinforces the role of FUS in neurodegeneration and will elucidate novel molecular mechanisms that could be druggable for ALS patients.

2090: **Transmissibility of SOD1 prion strains between mice expressing different mutant human SOD1s.**

Elaheh Ekhtiari Bidhendi 1, Peter M. Andersen 2, Stefan L. Marklund 1, Thomas Brännström1.
1 Department of Medical Biosciences, Umeå University, SE-90186 Umeå Sweden.
2 Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-90185 Umeå Sweden.

Background. Two structurally different human (h) SOD1 aggregate strains, A and B, can arise in CNS of transgenic (Tg) mice expressing hSOD1 variants (Bergh et al. 2015). When inoculated into spinal cords of Tg mice both strains transmit exponentially growing templated hSOD1 aggregation and premature paralysis (Bidhendi et al. 2016, 2018).
It is known from the prion protein field that there are species barriers for prion transmission and that mutations/polymorphisms in a given species can determine susceptibility to prion transmission. Here we investigate if such transmission barriers exist for hSOD1 aggregate/prion strains when prepared from, and inoculated into Tg mouse expressing different hSOD1 variants.
Methods. hSOD1 A and B seeds were prepared from mouse spinal cords by ultracentrifugation through a density gradient (Bidhendi et al. 2016). Seeds were microinoculated into lumbar spinal cord of adult recipient Tg mice.
Results. We have shown that A-prions prepared from hSOD1G85R, hSOD1G127X Tg mice and B-prions from hSOD1D90A mice efficiently seed aggregation and motor neuron disease in hSOD1G85R mice (Bidhendi et al. 2016, 2018).
We have also found that strain A seeds from hSOD1G85R mice efficiently seed aggregation and disease in both hemi and homozygous hSOD1D90A mice. Likewise strain B seeds from hSOD1D90A mice transmit disease to hemi and homozygous hSOD1D90A mice. A second passage strain B seed from hSOD1G85R mice transmitted disease to hSOD1G85R mice, but without any enhanced efficiency compared with such seeds from hSOD1D90A mice. Finally, strain A-like seeds from hSOD1G127X mice efficiently transmitted disease to both hemi and homozygous hSOD1G127X mice (unpublished data).
However, strain A seeds from hSOD1G93A Tg mice did transmit strain A aggregation and disease to hSOD1G85R mice, but apparently with low efficiency. The lifespans of recipient mice were longer than expected from the dose of hSOD1G93A aggregates that was inoculated. Finally, strain A seeds prepared from paralytic hSOD1WT mice (Graffmo et al. 2013) have so far failed to transmit disease to hSOD1G85R mice.
Conclusions. Our experience suggests that hSOD1 A and B prions on different hSOD1 mutation backgrounds in most cases freely transmit disease to Tg mice expressing other mutant hSOD1s. However, strain A seeds on hSOD1G93A background apparently showed reduced efficiency in hSOD1G85R mice, and we have so far failed to transmit ALS with strain A seeds from hSOD1WT Tg mice.

2096 : **Exogenous recombinant FUS is able to accumulate in cortical neurons in mouse brain.**

Perez M (1) Magdalini Polymenidou (2), Simon Alberti(3), Dupuis L (1).
1. INSERM U1118 : Mécanismes centraux et périphériques de la neurodégénérescence, Faculté de Médecine, Strasbourg, France

2.Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
3.Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease which affects primarily upper and lower moto-neurons, leading to paralysis and death. At least 5% of ALS cases display family history, and mutations in five major genes (TARDBP, FUS, SOD1, TBK1 and C9ORF72). ALS cases show typical proteinopathy with TDP-43 (encoded by TARDBP gene) aggregates as the major lesion found. In ALS-FUS cases, the FUS protein is cytoplasmically mislocalized and forms aggregates, and such pathology is also found in a significant proportion of patients with fronto-temporal dementia. Recent pathological work suggested that TDP-43 aggregates propagate through monosynaptic tracts from motor cortex to subcortical regions. This "prion-like" hypothesis of ALS propagation is consistent with clinical and imaging data [1]. While TDP-43 recombinant fibrils could be transmitted in vitro in cultured neurons, no evidence that such spreading of protein aggregates occurs in vivo has been provided. Furthermore, there exists, to our knowledge, no evidence of FUS proteinopathy propagation.

The goal of the present study is to investigate this "prion-like" hypothesis for FUS proteinopathy. To this end, we produced recombinant FUS-GFP protein that formed insoluble FUS-GFP fibrils after 3 days of incubation at room temperature [2]. Stereotaxic injections of minute amounts of recombinant FUS-GFP in the cerebral cortex of wild type mice demonstrated that these fibrils persisted at injection sites up to three months post injection. GFP- immunoreactivity was consistently observed in NeuN positive cortical neurons at 3 dpi, suggesting that exogenous FUS is able to penetrate in mouse neurons in vivo. Injection of FUS-GFP in the hippocampus led to strong accumulation of exogenous FUS in axonal tracts 3 dpi. One month and three months after injection, GFP immunoreactivity was much weaker, and mostly restricted to acellular aggregates in both cortex and hippocampus. Importantly, similar results were obtained in wild type mice and in knock-in mice expressing a truncated, partially cytoplasmic endogenous FUS [3]. FUS immunoreactivity overlapped mostly with GFP immunoreactivity. We are currently exploring the underpinning mechanism for neuronal intake of exogenous FUS through in vitro experiments in SH-SY5Y cells.

Funded by the ARSLA (call 2016).

[1] Braak H, Brettschneider J, Ludolph AC, et al.Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. Nat Rev Neurol. 2013 Dec;9(12):708-14./ [2] Patel A, Lee HO, Jawerth L, et al. A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell. 2015 Aug 27;162(5):1066-77./ [3] Scekic-Zahirovic J, Oussini HE, Mersmann S, et al. Motor neuron intrinsic and extrinsic mechanisms contribute to the pathogenesis of FUS-associated amyotrophic lateral sclerosis. Acta Neuropathol. 2017 Jun;133(6):887-906.

POSTER SESSION 2 THURSDAY 17.30-19.00

EPIDEMIOLOGY:

1970: **Application and optimisation of ensemble methods for survival analysis in ALS.**

Harry Bowles*(1,2,3), Alfredo Iacoangeli (1,2), Jiarui Liang (1,2), Ahmad Al Khleifat (2), Sarah Opie-Martin (2), Richard J Dobson (1,4,5), Stephen J Newhousea (1,4,5), Ammar Al-Chalabi (2,6).
1 Department of Biostatistics and Health Informatics, King's College London, UK.
2 Maurice Wohl Clinical Neuroscience Institute, King's College London, UK
3 NIHR Biomedical Research Centre Guy's and St Thomas' NHS Foundation Trust and King's College London, UK.
4 Farr Institute of Health Informatics Research, UCL Institute of Health Informatics, University College London, UK.
5 NIHR Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London, UK.
6 King's College Hospital, Bessemer Road, London, UK.

ALS progression is highly variable between patients and difficult to predict; the average survival time is 3 years following diagnosis but 5% of patients will survive for 20 years. A big-data approach may be the only way to capture this variation and develop models for survival prediction that generalise to all patients.
Machine learning (ML) methods are now mainstream, they are the subject of intense speculation within the scientific community and ML in medicine is becoming a pressing ethical issue. Here, we outline an attempt to develop a ML based survival analysis system for ALS patients using patient data from a range of sources including project MinE, Strength, Euromotor, national register and ALSoD.
Participant survival times were binned into three classes (0-25 months, 25-60 months, 60+ months), the training variables used to predict survival class included age of onset, diagnostic delay, presence of FTD and site of onset. A gradient boosting classifier (ensemble model) was chosen to model survival so that the most important training variables for prediction can be extracted – improving interpretability. Furthermore, previous unpublished research showed that this classifier performed better than alternatives, such as random forests, when trained in a similar context.
A custom script for cross-validated hill climbing was developed to optimise model hyper-parameters.
We had data from 2835 cases for analysis including 1707 males and 1120 females, and ages of diagnosis that ranged from 19 to 92.
We observed that the most important variables for prediction were age of onset and diagnostic delay. When country of patient origin was included as a training variable, it was ranked as third most important – though its score was much smaller than diagnostic delay and age of onset. This could suggest that differences in diagnostic criteria/ general practice between countries bias data slightly.
This method had a strong accuracy score of 0.73 when predicting survival for short term survival patients (0-25 months). It showed a lower, but still promising, accuracy of 0.6 and 0.61 for mid and long-term survival patients respectively.
To improve the model, we are currently adding genetic data to the training variable set. Genetic experiments that interfere with mutant ALS genes have been shown to alter survival rates suggesting that genetic information may explain some of the variance in our data and increase model accuracy.

1979: **Investigation of genotype-phenotype relations and malignancy risk of specific FUS mutations in 186 genetic ALS patients**.

Marcel Naumann (1,2,4), Kevin Peikert (1,4), René Günther (1,2), A.J. van der Kooi (6), E. Aronica (7), A. Hübers (8), V. Danel (9), P. Corcia (9), F. Pan-Montojo (10), S. Cirak (11), G. Haliloglu (12), Albert C. Ludolph (8), A. Goswami (13), P. M. Andersen (14), J. Prudlo (3,4,15), F. Wegner (16), P. Van Damme (17), Jochen Weishaupt (8) , Andreas Hermann (1-5).
(1) Department of Neurology, Technische Universität Dresden, Dresden, Germany
(2) German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany
(3) German Center for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, 18147 Rostock, Germany
(4) Translational Neurodegeneration Section „Albrecht-Kossel", Department of Neurology, University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany
(5) Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, University of Rostock, 18147 Rostock, Germany
(6) Department of Neurology, Amsterdam UMC, Academic Medical Centre, Amsterdam Neuroscience, University of Amsterdam, Amsterdam, the Netherlands
(7) Amsterdam UMC, University of Amsterdam, Department of (Neuro)Pathology, Amsterdam Neuroscience, Amsterdam, The Netherlands
(8) Department of Neurology, German Center for Neurodegenerative Diseases, University of Ulm, Germany
(9) Centre expert pour la SLA et les maladies du motoneurone hôpital SALENGRO, CHU, Lille, France
(10) Department of Neurology, Klinikum der Universität München, and Munich Cluster for Systems Neurology, SyNergy, 81377 Munich, Germany.
(11) Division of Pediatric Neurology, Department of Pediatrics, University Hospital Cologne, Cologne, Germany. And Center for Molecular Medicine, University of Cologne, Cologne, Germany
(12) Department of Pediatric Neurology, Hacettepe University Children's Hospital, 06100 Ankara, Turkey
(13) Institute of Neuropathology, RWTH Aachen University Hospital, Pauwelsstrasse-3052074, Aachen, Germany
(14) Institute of Pharmacology and Clinical Neuroscience, Umeå University, SE-90185 Umeå, Sweden.
(15) Department of Neurology, University of Rostock, Rostock, Germany.
(16) Department of Neurology, Hannover Medical School, Hannover, Germany
(17) Department of Neurology, University Hospitals Leuven; Department for Neuroscience, VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium.

Mutations in Fused in Sarcoma (FUS or TLS) are the fourth most prevalent in Western European familial ALS populations and have been associated with causing both, early and very late disease onset. FUS aggregation, DNA repair deficiency and genomic instability are contributors to the pathophysiology of FUS-ALS, but their clinical significance per se and their influence on the variability has yet to be sufficiently investigated. The aim of this study was to analyse genotype-phenotype correlations and malignancy rates in FUS-ALS patients.
We cross-sectional reviewed FUS-ALS patient histories in a multicenter cohort with 36 novel cases and did a meta-analysis of additionally 150 published FUS-ALS cases reporting the largest genotype-phenotype correlation of FUS-ALS.
Young age of onset (median 39 years, range 11-80) was correlated with shorter duration. C-terminal domain mutations were found in 90%. Among all, P525L and truncating/ frameshift mutations most frequently caused juvenile onset, rapid disease progression and atypical ALS while the R521 mutation site was associated with late disease onset and pure spinal phenotype. FUS-ALS patients did not show an increased occurance of malignancies
The data presented here allow for a prediction of the clinical course in newly diagnosed patients and their families.

Acknowledgements: We acknowledge all partners from the German ALS/MND NET and the team who helped in the collection of ALS tissue samples (Prof. dr. D. Troost, Prof. dr. M. de Visser, Dr. A.J. van der Kooi and Dr. J. Raaphorst
Funding: This work was supported in part by the NOMIS foundation to A.H., the Helmholtz Virtual Institute "RNA dysmetabolism in ALS and FTD (VH-VI-510)" to A.H., A.C.L., P.M.A., an unrestricted grant by a family of a deceased ALS patient to A.H, the Stiftung zur Förderung der Hochschulmedizin in

Dresden to A.H. and the Hermann und Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband to A.H. ; the Netherlands ALS foundation ("The Dutch ALS Tissue Bank") to AE.

2015: **Exploring the effect of Type II Diabetes Mellitus on ALS through Mendelian Randomization Analysis and review of published population-based studies.**

Gorka Fernández-Eulate (1, 2), Sarah Martin (2), Alfredo Iacoangeli (2), Aleksey Shatunov (2), Ahmad Al Khleifat (2), Anna Kulka (2), Ashley Jones (2), Isabella Fogh (2), Petroula Proitsi (2), Adolfo López de Munain (1), Ammar Al-Chalabi (2).
(1) Department of Neurology, Hospital Universitario Donostia, San Sebastián, Spain.
(2) Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK.

Background: There is evidence suggesting that insulin plays a role in normal brain function and that Type II Diabetes Mellitus (T2DM) and some neurodegenerative diseases share common pathways, although genomic loci of T2DM in genome-wide association studies (GWAS) have not been found to play a major role neurodegenerative diseases. T2DM and other cardiovascular risk factors have been associated inconsistently with a lower risk of ALS in population-based studies. We aimed to test the association and causality between T2DM and ALS using Mendelian Randomization (MR) analysis on the summary results of GWAS, as well as the effect of other cardiovascular risk factors associated with T2DM.
Material and methods: Single nucleotide polymorphisms (SNPs) specifically associated with T2DM extracted from a metanalysis of several GWAS (26,000 cases and 84,000 controls) were used as instrumental variables, and ALS project MinE data as outcome in a MR analysis using MR base. A multivariable MR analysis was performed using SNPs from hyperlipidemia and obesity GWAS. Power calculation was performed with mRnd.
Results: A total of 25 SNPs were analyzed after quality control were only the SNP rs5215 (KCNJ11) has been itself associated with ALS. Pleiotropy and heterogeneity analyses were non-significant. Different MR methods showed a non-significant trend toward protection of T2DM on the risk of ALS with the exception of MR Egger analysis which was statistically significant (p=0.04). Interestingly in a multivariate MR analysis, total cholesterol and LDL cholesterol were associated with a higher risk of ALS as previously published, and obesity or triglycerides were non-significant. This makes us hypothesize that the weak T2DM protective effect on ALS reported on population-based studies does not come from an increase of cardiovascular risk factors. A systematic review in 2014 found a similar prevalence of T2DM in ALS patients and controls, and no effect on disease progression or survival patients. However, in a 2015 Danish population-based study, T2DM was found to decrease the risk of ALS, whereas obesity only showed a non-significant trend toward protection. This was also confirmed in a similar Italian study in 2018.
Conclusion: Further studies are needed to confirm a possible protective causal effect of T2DM on ALS. This relationship does not seem to be related with other cardiovascular risk factors commonly associated with T2DM.

2035: **Investigation of ALS risk using the Comprehensive Smoking Index**.

Sarah Opie-Martin*(1), Pamela J Shaw(2) , Christopher E Shaw (1), Neil Pearce (3), Karen E Morrison(4), George Davey-Smith(5), Gibran Hemani(5), Ashley Jones(1), Ammar Al-Chalabi(1).
(1) Maurice Wohl Clinical Neuroscience Institute, King's College London, Department of Basic and Clinical Neuroscience, London, United Kingdom.
(2) Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom.
(3) London School of Hygiene and Tropical Medicine, London, UK.
(4) Faculty of Medicine, University of Southampton, UK .

(5) MRC Integrative Epidemiology Unit at the University of Bristol, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, UK.

Introduction: Higher susceptibility to amyotrophic lateral sclerosis (ALS) has been associated with smoking exposure in some studies. It is not clear what measure of smoking exposure is associated with ALS and whether it is causal.
The comprehensive smoking index (CSI) is a model of lifetime smoking exposure that combines duration, intensity and time since cessation into a single score. The score can be used as a covariate representing lifetime smoking exposure in a regression model. This allows all factors to be considered when investigating smoking, avoiding potential issues of multicollinearity between smoking exposure variables. This measure has not been used to investigate the role of smoking in ALS risk before. We perform a retrospective case control study and Mendelian Randomisation to investigate the relationship.
Methods:Smoking status was collected using environmental questionnaires from people diagnosed with ALS between 2008 and 2013 at three centres in the UK, and from age, sex and geographically matched controls. We ran simulations to estimate optimal values of tau and delta, the parameters needed to construct the CSI score. Delta, also referred to as half-life, reflects the exponential decay in the effect of smoking on health outcomes during lifetime. Tau, or lag-time, reflects that smokers may be at a higher risk of disease immediately after quitting due to reverse causality.
Logistic regression models were constructed to assess risk of ALS with CSI score and adjusted for age at survey, gender, level of education, smoking status and alcohol initiation.
Two-sample mendelian randomisation using GWAS summary data was performed to investigate causality. SNPs associated with higher CSI in UK Biobank data were used as an instrumental variable to represent smoking exposure, a recent GWAS study of ALS was used as the outcome dataset.
Results: 388 records with full smoking history were available for case-control analysis. The optimal CSI variables were tau = 2 and delta = 3.6. Increase in value of CSI did not increase risk of ALS: OR 0.806 (95% CI 0.576-1.11) p-value 0.2. Current smoking increased risk of ALS, OR 3.63 (95% CI 1.02-13.9) p-value=0.05.
Using Mendelian randomisation to investigate whether lifetime smoking level is causal of ALS we found no association.
Discussion: There is a weak positive effect of current smoking increasing the risk of ALS however we do not find this is dose dependent with higher levels of lifetime smoking.

GENOMICS:

1901: **Rare variants in UNC13A and SCA31 may risk Amyotrophic Lateral Sclerosis.**

Ton Fang (1*), Aleksey Shatunov (1), Ammar Al-Chalabi (1).
1. King's College London, Maurice Wohl Clinical Neuroscience Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, De Crespigny Park, London, SE5 8AF, UK.

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of upper and lower motor neurons, leading to eventual death through respiratory failure. Increasingly evidence suggests that ALS develops in a multi-step fashion, where excessive genetic and environmental insults compound to exceed threshold for disease. As many as 40 genes are associated with ALS, with variations in genetic sequences of UNC13A and SCA31 shown to affect survival in this disease. Identifying these genetic variations can provides useful insights into ALS mechanisms and in turn, common molecular pathways for therapeutic targets.
Methods: Next generation sequencing (NGS) was used to identify rare single nucleotide polymorphisms (SNPs) on high risk ALS alleles. SNPs found on UNC13A and SCA31 genes were confirmed using Sanger sequencing technique. DNA was replicated using PCR and products confirmed using electrophoresis. Sequencing was completed by Source Bioscience. Sequence data was analysed to identify genotype and rarity of confirmed SNPs.
Findings: From 700 ALS patients in the UK cohort, there were 26 SNPs identified on UNC13A and SCA31 through NGS. 15 heterozygous mutations were confirmed using Sanger technique, 7 for UNC13A and 8 for SCA31, with no mutations in the 41 controls. SCA31 and UNC13A rare variants accounted for ~1% of ALS patients and was over 25 and 100 times the risk of baseline population.
Interpretation: Rare variant on UNC13A was seen in the promoter region upstream to Exon 1, a region thought to be responsible for mitochondrial function. Disruption to the regulation of this region may lead to interruption of cellular survival and neuronal apoptosis. Less is understood about the region affected in SCA31. Future studies should focus on understanding the effects of these mutations in cellular and animal models, in helping to understand molecular pathways to target for in ALS.

1972: **Genetic and functional analysis of TIA1 in a large cohort of FALS and patients with early onset ALS.**

François Muratet (*) (1), Elisa Teyssou (1), Justine Guégan (1), Agnès Rastetter (1), Delphine Bouteiller (1), Yannick Marie (1), Ludmila Jornea (1), Delphine Bohl (1), Christian Lobsiger (1), Séverine Boillée (1), Danielle Seilhean (1,3), François Salachas (1,2), Nadine Le Forestier (2,4), Maria-Del-Mar Amador (2), Stéphanie Millecamps (1).
(1) Institut du Cerveau et de la Moelle épinière, ICM, Inserm U1127, CNRS UMR7225, Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France.
(2) Centre de référence Maladies rares SLA-IDF, département de Neurologie, Hôpital Universitaire Pitié-Salpêtrière, Paris, France.
(3) Département de Neuropathologie, Hôpital Universitaire Pitié-Salpêtrière, Paris, France.
(4) Département de recherche en éthique, Université Paris Sud/Paris Saclay, Orsay, France.

Progress in genetics accelerated the discovery of causative genes in Amyotrophic Lateral Sclerosis (ALS) and about 25 genes have now been incriminated in the disease. Mutations in TIA1 have recently been identified in patients with familial or sporadic forms of the disease. This gene is also known as a cause of Welander Distal myopathy (WD), mostly affecting the upper distal muscles and characterized in some cases by the presence of rimmed vacuoles in muscular biopsy. Mutations identified in ALS or WD patients were all located in the C-terminal prion-like domain of the protein. Other genetic studies performed on cohorts of

ALS patients did not find any TIA1 mutation and additional analyses are needed before considering TIA1 as a genetic cause of the disease. TIA1 encodes a protein involved in the formation of stress granules, small and transient compartments allowing the protection of mRNA in cells exposed to stress conditions. To confirm the contribution of TIA1 to ALS disease, we performed exome analyses of 150 FALS (devoid of mutation in any major ALS genes) and 80 ALS patients with early onset of the disease (including 20 trios) as genetic causes could be preponderant in these patients. We identified a c.653G>A, p.Cys218Tyr (C218Y) variant, located in a RNA-recognition motif of the protein, in a female patient who started ALS at 30 years. The analysis of the trio showed that this variant was transmitted by her healthy father. This variant was absent from gnomAD control database, affected a residue that is conserved among species (including Zebrafish) and was predicted to be deleterious by in silico analyses (SIFT, MutationTaster, Polyphen-2 and Panther) and by a pathogenic CADD phred score at 29. Analysis of fibroblasts from this patient showed round TIA1-positive inclusions, some of which were also positive for p62, one of the neuropathological hallmarks of ALS disease detected in post-mortem motor neurons. Similar inclusions were also positive for TDP-43 but remained negative for FUS. Our future directions will evaluate the dynamics of stress granules after exposing the fibroblasts to stress conditions and CRISPR/Cas9 gene editing technology will be used to determine whether these phenotypes can be rescued by suppressing the expression of this TIA1 mutation. Overall these results will help to conclude about the pathogenicity of this novel TIA1 mutation.

2025: **Mutations within the glycosyltransferase domain of GLT8D1 cause familial amyotrophic lateral sclerosis.**

Tobias Moll*(1), Johnathan Cooper-Knock (1,12) Tennore Ramesh (1), Lydia Castelli (1), Alexander Beer (1), Henry Robins (1), Ian Fox (1), Isabell Niedermoser (2), Philip Van Damme (3,4), Matthieu Moisse (3), Wim Robberecht (3,4), Orla Hardiman (5), Monica P. Panades (6), Abdelilah Assialioui (6), Jesus S. Mora (7), A. Nazli Basak (8), Karen E. Morrison (9), Christopher E. Shaw (10), Ammar Al-Chalabi (10), John E. Landers (11), Matthew Wyles (1), Paul R. Heath (1), Adrian Higginbottom (1), Theresa Walsh (1), Mbombe Kazoka (1), Christopher J. McDermott (1), Guillaume M. Hautbergue (1), Janine Kirby (1) and Pamela J. Shaw (1).
1. Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK.
2. Department of Molecular Evolution and Development, University of Vienna, Vienna, Austria.
3. VIB-KU Leuven Center for Brain & Disease Research, KU Leuven, Leuven, Belgium.
4. University Hospitals Leuven, Department of Neurology, Leuven, Belgium.
5. Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.
6. Hospital Universitari de Bellvitage, Barcelona, Spain.
7. Hospital San Rafael, Madrid, Spain.
8. Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey.
9. Faculty of Medicine, University of Southampton, Southampton, UK.
10. Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.
11. University of Massachusetts Medical School, Worcester, USA.

Rationale & Hypothesis: ALS-causing mutations were recently identified within the gene encoding the glycosyltransferase GLT8D1. Exome sequencing in an autosomal dominant ALS pedigree identified p.R92C mutations in GLT8D1 which co-segregate with disease. Sequencing of local and international cohorts identified 8 additional patients with the p.R92C variant and a single patient with a p.G78W variant. We hypothesise that GLT8D1 mutations cause motor neuron loss in ALS via a new disease pathway.
Objectives: To develop cellular and animal models to characterise the pathological pathway linking GLT8D1 variants (p.R92C and p.G78W) to the loss of motor neurons.
To identify novel therapeutic targets for the treatment of ALS.

Methodology: Immunocytochemistry was used to assess the intracellular localisation of wild-type and mutant GLT8D1. The toxicity of GLT8D1 mutations in N2A and HEK-293 cells was assessed by MTT and lactate dehydrogenase assays. Knockdown of endogenous GLT8D1 and overexpression of wild-type and mutant GLT8D1 in zebrafish embryos was performed via microinjection of morpholino and mRNA, respectively. Enzyme activity of purified wild-type and mutant GLT8D1 was measured using a UDP-Glo glycosyltransferase assay. Fluorescent probes targeting sialic acid residues in isogenic HEK-293 cells expressing wild-type and mutant GLT8D1 were imaged live using confocal microscopy.
Findings: Immunocytochemistry showed localisation of GLT8D1 to the Golgi network in N2A and HEK-293 cells. When compared to wild-type GLT8D1, mutant forms are cytotoxic and reduce cellular metabolism. The p.R92C mutation was more toxic than p.G78W in all assays. Knockdown of endogenous GLT8D1 and overexpression of mutant GLT8D1 produced a motor phenotype in zebrafish embryos at 5 days post-fertilisation. p.R92C and p.G78W variants reduced the enzymatic activity of GLT8D1. Initial observations suggest a mutation-specific reduction in membrane sialic acid fluorescence intensity, however quantification is still underway and will be available for presentation.
Discussion: The relative toxicity of GLT8D1 mutations in model systems mirrors clinical severity and our findings are consistent with loss-of-function toxicity. ALS pathophysiology is linked to inherited mutations that diminish the activity of a glycosyltransferase enzyme.

2026: **Processing the Project MinE Data on the SURFsara Grid.**

Brendan J Kenna, Maarten Kooyman, Kristel van Eijk, Joke van Vught, Kevin P Kenna, Jan Veldink. Department of Neurology Brain Centre, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, the Netherlands.

All Project MinE genetic data is stored, processed and analyzed on the SURFsara grid that is located in the Amsterdam Science Park in the Netherlands. In order to improve the statisitcal power of burden testing and other experiments, the Project MinE genetic data is actively being merged with genetic data from external biobanks. In order to assess the utility of merging the Project MinE data with these external datasets, basic metrics such as overall sample quality and global ancestry are calculated during the initial data processing. By creating separate applications for each pipeline such as merging the raw biobank data or calculating the sample summary metrics, we can quickly and efficiently process the Project MinE data so that the petabyte scale storage & compute resources offered by the SURFsara grid is fully utilized.

2094: **The role of non-LTR retrotransposons in the increased genetic burden to MND at the NEK1 gene.**

Jack N. G. Marshall1, Gerald G. Schumann2, Abigail L. Savage1, Richard J. Mead3, Pamela J. Shaw3, Vivien J. Bubb1 and John P. Quinn1.
1 Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool.
2 Division of Medical Biotechnology, Paul-Ehrlich-Institut, Langen, Germany.
3 Department of Neuroscience, Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield.

Genome-wide association studies (GWAS) and functional data has shown that there is a genetic basis contributing to sporadic and familial forms of MND. We have focused on the non-LTR (non-Long Terminal Repeat) retrotransposons, representing transposable elements in the human genome known to be currently mobilised in the brain and central

nervous system (CNS) as genetic elements affecting the risk to MND. This subclass is divided into three families: Long Interspersed Elements-1 (LINE-1, L1), Alu elements and SVA (SINE-VNTR-Alu) elements Using whole genome sequencing (WGS) data and retrotransposon capture sequencing (RC-Seq) it has been possible to detect non-LTR retrotransposon presence or absence insertion polymorphisms which previously had not been identified. Non-LTR retrotransposon can affect gene expression by two distinct mechanisms; as regulatory elements in the germline (found in the reference genome sequence) and as source elements for insertional somatic mutation via their mobilisation. We have focused on defining the non-LTR retrotransposon genetic variation generated by 1) presence or absence polymorphism of these elements and 2) polymorphism within the non-LTR retrotransposon itself, to determine the increased genetic burden at the NEK1 locus. We will present data on the frequency of such elements in MND cohort and how these domains may modify NEK1 gene expression.

MECHANISMS:

1904: **Neuroanatomical quantitative proteomics reveals pathogenic biological routes in amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD).**

Marina Oaia Iridoy*(1), Leyre Martínez (1), Irene Zubiri (2), Maria Victoria Zelaya (3), Karina Ausin (2), Mercedes Lachen-Montes (2), Enrique Santamaría (2), Joaquin Fernández-Iridgoyen (2), Ivonne Jericó (1).
1: Departament of Neurology Complejo Hospitalario de Navarra. Pamplona. Spain.
2: Proteored-ISCIII Proteomics Unit Navarrabiomed. Pamplona. Spain.
3: Pathological Anathomy Service Complejo Hospitalario de Navarra. Pamplona. Spain.

Introduction: Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FDT) are neurodegenerative disorders with an overlap in clinical presentation and neurophatology. The present study was conceived to perform a deep proteomic analysis of the spinal cord from ALS patients and DFT patients comparing them with post mortem tissue from non-neurodegenerative control donors. Methods: A liquid chromatography tándem mass spectometry (LC-MS/MS) analysis of the spinal cord from ALS subjets, ubiquitin -positive frontotemporal lobar dementia subjetcts (FTLD-U) and neurological intact controls was performed. Results: 281 differentialy-expressed proteins were detected among ALS versus control subjets, while 52 proteins were deregulated among FTLD-U cases and the control group. The resulting data was subjected to network-driven proteomics analysis, revealing mitocondrial dysfunction and metabolic impairment, both for ALS and DFTL-U that could be validated throught the confirmation of expression levels changes of the Prohibitin (PBH) complex. Conclusions: ALS and DFTL-U share molecular and functional alterations, althought part of the proteostatic impairment is region and disease specific. We have confirmed the involvement of specific proteins previously associated with ALS (LGALS, TTR, S100A6, S100A11) and have shown the involvement of proteins not previously described in the ALS context (SELENBP1, PIN-1, CACYBP and ROCK2).

1914: **Antiviral immune response as a trigger of FUS proteinopathy in amyotrophic lateral sclerosis**.

Haiyan An1, Vladimir L. Buchman1 and Tatyana A. Shelkovnikova1,2*.
1 Biomedicine division, School of Biosciences, Cardiff University, Cardiff, CF10 3AX, United Kingdom
2 Medicines Discovery Institute, Cardiff University, Cardiff, CF10 3AT, United Kingdom.

Mutations in the fused in sarcoma (FUS) gene cause a subset of familial amyotrophic lateral sclerosis cases (ALS-FUS). Mutant FUS mislocalises to the cytoplasm of neurons and glia, where it is eventually deposited in a form of insoluble inclusions, thus marking the onset of FUS proteinopathy. Mutant FUS is highly affine to cytoplasmic RNA granules stress granules (SGs), and can also form small RNA granules spontaneously which grow under conditions of stress. It is believed that stress-induced mutant FUS assemblies may become precursors of pathological inclusions, however the nature of stress which acts as a trigger for FUS proteinopathy is still elusive. To address this, we characterised the ability of known SG-inducing stresses to cause sustained presence of mutant FUS-enriched cytoplasmic assemblies. Using CRISPR/Cas9 cell lines expressing endogenous mutant FUS we found that a viral infection mimic, synthetic double-stranded (ds) RNA poly(I:C), is a stressor whose single application is sufficient to cause formation of cytoplasmic FUS-containing assemblies which persist in cells for up to 48 hours. These FUS assemblies sequester the autophagy receptor optineurin and nucleoplasmic transport factors including FUS import receptor, Transportin 1. Moreover, mutant FUS expressing cells, including patient fibroblasts, are hypersensitive to dsRNA toxicity. Finally, we found that an integral

component of the antiviral immune response, type I interferon, promotes FUS protein accumulation by increasing its mRNA stability. Our data suggest that antiviral immune response can expedite the onset and progression of FUS proteinopathy by promoting FUS protein accumulation and its coalescence into persistent cytoplasmic aggregates.

1915: **A novel pathogenic mechanism of ALS-associated VCP-mutant.**

V.Ferrari (*)(1), M.E. Cicardi (2), P. Rusmini (1), V. Crippa(1), R. Cristofani(1), B. Tedesco(1), M. Galbiati(1), E. Messi(1), M. Piccolella(1), A. Poletti(1).
(1) DiSFeB, UNIMI, MIlan, Italy.
(2) Department of Neuroscience, Sidney Kimmel Medical College - Jefferson University, Philadelphia, Pennsylvania (USA).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by motorneuron death. ALS is classified as a proteinopathy in fact, the alteration of proteinostasis is one of main pathogenic mechanism that is associated to the ALS. All ALS forms are characterized by the presence of insoluble protein aggregates in the brain tissue of effected patient. Moreover, there are many genes involved in protein regulation that are found mutated. One of these genes associated to ALS encodes for Valosin Containing Protein (VCP), an AAA+ ATPase. VCP has many roles in the regulation of proteinostasis. Recent studies demonstrate that VCP is also involved in the removal of altered organelles like lysosomes. The alteration of lysosomes is deleterious for cell; firstly, for its key role in proteinostasis and secondly lysosome-damage leads to massive lysosomal leakage that causes cell toxicity and death. There are different mechanisms that can be activated to maintain the lysosomal activity. The most studied is lysophagy where VCP has been found involved.
We have found that the overexpression of VCP wild type (WT) and fALS mutations (VCP R155H; VCP R191Q) decrease the levels of insoluble species of a SOD1-mutant (SOD1 G93A). In addition, we study lysosomal-damage response in presence of overexpressed VCP WT and its mutants in NSC-34 cell line. To study VCP contribute in the clearance of damaged lysosomes we used trehalose treatment that induces lysosome damage and in addition we found that the overexpression of SOD1 G93A causes lysosome damage. We observed that overexpressed VCP WT reduces lysosomal damage when it is induced by trehalose or by SOD1 G93A overxpression. On the contrary, VCP R155H induces lysosomal damage in basal conditions and prevents the clearance of damaged lysosomes when the damage is induced by trehalose. VCP R191Q also induces lysosome damage in basal conditions, but to lower rate compared to VCP R155H. When the damage is induced, we observed that VCP R191Q can partially reduce lysosomal damage, but it significantly loses its functionality compared to overexpressed VCP WT. Moreover, the overexpression of the VCP-mutants leads to the conversion of LC3-I into LC3-II that significantly increases when cells are treated with NH4Cl, a inhibitior of autophagy. This suggests an activation of autophagy in the presence of VCP-mutants. The activation of the autophagic could explain the decrease of insoluble aggregates when VCP-mutants are overexpressed.

1923: **Single cell transcriptomics of degenerating human motor neurons identifies master regulators of synaptic dysfunction in SOD1 ALS.**

Seema C. Namboori(1), Patricia Thomas(2), Ryan Ames(1,3), Lawrence O. Garrett(1), Craig R. G. Willis(4), , Lawrence W. Stanton(5,6), Akshay Bhinge(1,2)*.
1. Living Systems Institute, University of Exeter, EX4 4QD, UK.
2. University of Exeter Medical School, EX1 2LU, UK.
3. Centre for Biomathematical Modelling and Analysis, University of Exeter, EX4 4QD, UK.
4. Department of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, EX1 2LU, UK.

5. Genome Institute of Singapore, 138672, Singapore.
6. Current address: Humacyte, NC 27713, USA.

The "dying back" hypothesis postulates that degeneration is initiated at the motor neuron synapse before death of the neuron body. However, the mechanism driving this loss is poorly understood. To investigate this mechanism, we utilized single cell transcriptomics of degenerating MN derived from ALS patients to uncover key transcriptional drivers of dysfunctional pathways in ALS. We developed an in vitro human model of ALS based on differentiating SOD1 ALS iPSC into spinal MN. CRISPR-Cas9 genome corrected iPSC were used as isogenic controls to causally link the molecular changes to the observed degenerative phenotypes. Single cell analysis of spinal neurons derived from ALS and isogenic iPSC allowed us to classify cells into neural subtypes including MN and interneurons (IN). Differential expression analysis between disease and control MN revealed downregulation of genes involved in synaptic structure, neuromuscular junction, neuronal cytoskeleton and mitochondrial function. Interestingly, IN did not show suppression of these homeostatic functions. Single cell expression data enabled us to derive an ALS-specific transcriptional network. Master regulator analysis on this network identified core transcriptional factors driving the ALS gene expression signature. Specifically, we were able to correlate suppression of HOX genes to synaptic dysfunction in ALS MN. Additionally, genome-wide enhancer mapping using H3K27Ac ChIP-sequencing corroborated involvement of HOX genes in ALS. Strikingly, we also found suppression of HOX genes by mutant SOD1 in lumbar MN of a mouse model of ALS, suggesting that HOX gene inhibition may be a general phenomenon in SOD1 ALS. We propose that ALS associated SOD1 mutation leads to inhibition of transcriptional networks driving motor neuron homeostatic programs. Our results demonstrate the utility of single cell transcriptomics in mapping MN-specific networks driving neurodegeneration in ALS.

1981: **Translating ribosome affinity purification from C9orf72-ALS/ FTD patient-derived iPSC-Motor Neurons.**

Chaitra Sathyaprakash* (1), Jakub Scaber (1), Nidaa Ababneh (1), Ana Candalija (1), Ruxandra Dafinca (1), Kevin Talbot (1).
(1) Nuffield Department of Clinical Neuroscience, University of Oxford, Oxford, United Kingdom.

Background: The C9orf72 hexanucleotide repeat expansion is the commonest cause of amyotrophic lateral sclerosis (ALS) in both sporadic and familial patients. Motor neurons differentiated from patient-derived induced pluripotent stem cells (iPSCs) are a powerful model for studying the pathways involved in ALS, since they exhibit several robust pathological phenotypes. However, transcriptomic analysis of these motor neurons is challenging due to multiple sources of experimental variance, such as the presence of up to 30% of cells in culture being of unknown identity.
Aims: The aim of this project is to use translating ribosome affinity purification (TRAP) to isolate an enriched population of RNA from human iPSC-motor neurons, to achieve a more accurate analysis of the translatome profile of this model. We will examine differential gene expression and splicing profiles between control and C9/ALS motor neurons.
Methods: We expressed a lentiviral TRAP vector with a large ribosomal subunit protein, RPL22, fused to an affinity tag, and bicistronic enhanced green fluorescent protein, under a choline acetyl transferase (ChAT) promoter in our iPSC-motor neurons. Magnetic beads were used to pull down tagged RPL22-containing polysomes from motor neuron lysates to isolate RNA. Following quality control and library preparation, samples were submitted for Illumina next generation RNA sequencing.

Results: Optimisation of the TRAP protocol at several steps has been crucial to reliably obtain enriched RNA. We have achieved up to 100% co-expression of the eGFP reporter and ChAT in control and patient iPSC-motor neurons, transduced with TRAP lentivirus. Quantitative-RT-PCR of TRAP-RNA showed enrichment of ChAT and RPL22-tag transcripts compared with their corresponding input fractions. Immunoblotting showed the expression of both endogenous and tagged RPL22. Data analysis has revealed differences in expression profiles between TRAP-RNA and input fractions. Gene set enrichment analysis will be used to identify over-represented gene families, and their linked pathways associated with early changes of neurodegeneration in C9orf72 motor neurons.
Conclusions: Analysis of the C9/ALS patient translatome, in conjunction with proteomic analysis is anticipated to overcome some of the problems currently associated with the variation in transcriptomic outputs from iPSC models.
Funding: The Barry Bastin PhD Studentship (Motor Neurone Disease Association) and All About Alie Charity.

1928: **Disruption of nucleocytoplasmic transport in SOD1 ALS.**
Shirel Argueti, Joy kahn, Adrian Israelson.
Dept. of Physiology and Cell Biology, Ben-Gurion University of the Negev, P.O.B. 653 Be'er-Sheva 84105, Israel.

Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease caused by the progressive loss of motor neurons in the brain and spinal cord. Nucleocytoplasmic transport (NCT) dysfunction has been implicated as a possible target for toxicity by several studies in C9ORF72, TDP43 and FUS-related ALS as well as in other neurological diseases. However, whether misfolded SOD1, which accumulates in the motor neurons, affects this pathway, is still unknown. The NCT pathway is essential for maintaining cell viability; transport of proteins and nucleic acids between the nucleus and the cytoplasm. Our data using a shuttle-GFP reporter, show that misfolded/mutant SOD1 disrupts the nuclear pore normal function in regulating active import/export from the nucleus in a human neuronal cell line (SH5Y5). Following Zhong and colleagues finding, misfolded SOD1 exposes an NES-like sequence, which results in its export from the nucleus via recognition of CRM1. We hypothesize that this association and export of misfolded SOD1 from the nucleus could trigger its contribution to the NCT disruption. Indeed, we showed that SH5Y5 cells transfected to express the double mutant SOD1G93A/L38R, which affects the NES-like sequence and binding to CRM1, are protected from NCT disruption. Moreover, we revealed that CRM1 and Ran-Gap1, another key regulator of NCT, are sequestered and mislocalized in the spinal cord of symptomatic mutant SOD1 transgenic mice and mutant SOD1 expressing SH5Y5 cells, respectively. Finally, we show that macrophage migration inhibitory factor)MIF) chaperone function can rescue the NCT disruption caused by misfolded SOD1, and suggest it may serve as a potential therapeutic candidate for mutant SOD1 toxicity in ALS.

1931: **Overexpression of MIF rescues mutant TDP-43 mislocalization in a cellular model**.
Adiva Charikar*, Joy Kahn and Adrian Israelson.
Department of Physiology and Cell Biology, Faculty of Health Sciences and the Zlotowski Center for Neurosciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by the progressive loss of the upper and lower motor neurons. Hallmark proteins, such as mutant SOD1 and TDP-43, drive this degeneration, increase disease

susceptibility and its progression. Mutations in the genes encoding these proteins are found in familial cases of ALS. Wild type SOD1 and TDP-43 are also found in aggregates from sporadic ALS patients, supporting their widespread role in neuronal degeneration. Our laboratory has recently identified the multifunctional protein macrophage migration inhibitory factor (MIF) to directly inhibit mutant SOD1 misfolding and toxicity. Since TDP-43 mutants share similar mechanisms of toxicity with mutant SOD1 in ALS pathogenesis; we aimed to discover whether MIF, as it does with misfolded SOD1, affects the toxicity of mutant TDP-43 proteins.
Results: Wild type TDP-43 and four different mutants were successfully overexpressed with or without MIF in HEK-293T cells. Lysates from these cells were analyzed by western blot for protein expression. Surprisingly, cells co-expressing mutant TDP-43 and MIF resulted in reduced TDP-43 accumulation in their cytoplasmic fractions. These findings were further supported by immunocytochemistry and confocal microscopy analysis. Furthermore, supporting our results, a direct interaction between MIF and TDP-43 proteins was demonstrated using co-immunoprecipitation.
Conclusions: Our results suggest a rescue effect of MIF on TDP-43 mislocalization. Since cytosolic TDP-43 is known to be toxic to cells, thus, the possible sequestering of TDP-43 from the cytoplasm by MIF may rescue the cells from the toxic effects of the mutant protein. This may be achieved by shuttling of TDP-43 back to the nucleus, where it performs its main function. Alternatively, the presence of MIF may serve as a signal for cytoplasmic TDP-43 degradation. Further experiments are underway to elucidate the specific mechanism by which MIF elicits its protective effect on TDP-43 cellular distribution.

1936: **Deciphering the respective contribution of macrophages and microglia to motor neuron degeneration in ALS using human iPS cells.**

Elise Liu* (1), Cynthia Lefebvre (1), Stéphanie Bigou (1), Clément Daube (1), Jean-Michel Peyrin (3), Josquin Courte (3), François Salachas (1,2), Lucette Lacomblez (2), Christian Lobsiger (1), Stéphanie Millecamps (1), Séverine Boillée (1), Delphine Bohl (1).
(1) ICM- INSERM U 1127 - CNRS UMR-7225 –Sorbonne Université, Paris, France.
(2) APHP, Centre de ressources et de compétences SLA Ile de France, Hôpital Pitié-Salpêtrière, Paris, France.
(3) IBPS - CNRS UMR 8246/INSERM U 1130 - Paris cedex 05, France.

In Amyotrophic Lateral Sclerosis (ALS), disease initiation is likely intrinsic to motor neurons but its progression may involve trophic and/or toxic effects of myeloid cells present in the environment of the motor neurons. Since spinal motor neurons have their soma in the central nervous system (CNS) while their axons lay at the periphery in order to contact to muscles, it involves two distinct micro environments: in the CNS, the motor neuron soma is surrounded by microglia, the resident macrophages of the CNS, while at the periphery its axon is in surrounded by macrophages. To better understand their respective implication in ALS disease progression, we want to analyse the possible trophic and/ or toxic contributions of human macrophages and microglia to ALS motor neuron degeneration using new cellular models derived from human induced pluripotent stem cells (iPSc). iPSc clones from familial and sporadic ALS patients are already available in the lab. Moreover, protocols were established to obtain pure cultures of iPSc-derived spinal motor neurons, macrophages and more recently microglial cells. To decipher the specific physiological interactions between microglia and motor neuron soma, or macrophages and motor neuron axons, microfluidic devices are used. The inflammatory responses of ALS microglia and macrophages are currently investigated, as well as their mediated toxicity to motor neuron survival with mixed matched cultures with the different ALS mutations, isogenic controls and aged matched controls. On a more global, we are also in the process to realise RNA-seq analysis of ALS myeloid cells to identify affected target genes and inflammatory pathways,

which could help to define new therapeutic options. As ALS is diagnosed once symptoms are already present, acting on disease progression by modulating microglia/macrophages could benefit to all ALS cases.

1947 : **Modulating the reactive responses of peripheral macrophages to slow ALS progression.**

Félix BERRIAT* (1), Aude CHIOT (1), Matthieu RIBON (1), Danielle SEILHEAN (1)(2), Stéphanie MILLECAMPS (1), Delphine BOHL (1), Christian LOBSIGER (1), Séverine BOILLÉE (1)
1 Institut du Cerveau et de la Moelle épinière, ICM, Inserm U 1127, CNRS UMR 7225, Sorbonne Université, F-75013, Paris, France.
2 Département de Neuropathologie, AP-HP, Hôpital Pitié-Salpêtrière, Paris, France

It is now well established that non-neuronal cells are involved in the progression of amyotrophic lateral sclerosis (ALS). Pioneering studies have shown that reducing expression of toxic mutant SOD1 in specific non-neuronal cells could affect the lifespan of ALS mice. In particular, microglial cells in the central nervous system and peripheral macrophages in contact with the axons of the spinal motor neurons not only react to the death of motor neurons but also actively participate in neurodegeneration. However, these cells have distinct developmental origins and are very sensitive to their environment, which is why we hypothesized they could hold very distinct disease modifying capacities. The objective of this project is to decipher the respective contributions of microglia and peripheral macrophages to ALS progression and then to focus specifically on macrophages to identify therapeutic targets modulated throughout disease course. In a previous study, we used a specific bone-marrow grafting procedure (which does not negatively affect the blood-brain-barrier integrity) in the SOD1 mouse model of ALS to study the specific influence of the immune system at the periphery. This protocol had overall positive but very distinct impacts on disease progression, depending on the disease stage at which the graft was performed. Importantly, the replacement of peripheral macrophages (with cells less neurotoxic / more neurotrophic) at disease onset led to a significant increase of the mouse survival, which is very encouraging from a therapeutic point of view. To go further, we also performed a transcriptomic experiment over the entire course of the disease, which is currently being analysed. The preliminary data confirmed that microglia and macrophages have very distinct expression profiles over the disease course. The objectives are now to identify precise candidates in peripheral macrophages, and the best disease stage to target them, in order to render them less neurotoxic or more neurotrophic and thereby slow ALS disease progression.

1951: **Aggregation of TDP-43 protein in Amyotrophic Lateral Sclerosis (ALS) and sumoylation pathway.**

Cindy Maurel (1), Anna Chami (1), Rose-Anne Thépault (1), Sylviane Marouillat (1), Hélène Blasco (1,2), Philippe Corcia (1,3), Christian Andres (1,2), Patrick Vourc'h (1,2,*).
(1) UMR Inserm 1253, Université de Tours, IBrain, Team Neurogenomics and Neuronal physiopathology, Tours, France.
(2) CHRU de Tours, Hospital, Department of Biochemistry and Molecular Biology, Tours, France.
(3) CHRU de Tours, Hospital, Department of Neurology and ALS Center, Tours, France.

Aggregates positive for TDP-43 proteins are present in the cytoplasm of degenerating Motor neurons in ALS. TDP-43 protein is a DNA / RNA binding protein predominantly present in the nucleus of cells. We investigated the role of SUMOylation, a post-translational protein modification, in the formation of TDP-43 positive cytoplasmic aggregates and survival of motor neuronal cells.

We first inhibited the SUMOylation pathway by adding anacardic acid, an inhibitor of the first step of this enzymatic pathway. We showed that anacardic acid reduces the quantity of cells with TDP-43 positive aggregates, improves neuritogenesis and cell viability of motor neuronal cells in vitro. We next specifically inhibited the SUMOylation of the TDP-43 protein by the mutation of its unique potential SUMOylation site (lysine 136). We showed by immunoprecipitation the possible SUMOylation of TDP-43. Very interestingly, we observed that the modification of this lysine 136 (p.L1236R) of TDP-43 modifies its intracellular localization. Indeed, cells overexpressing wild-type TDP-43 showed cytoplasmic aggregates, whereas cells overexpressing mutant TDP-43 L136R contained nuclear aggregates. This re-localization of TDP-43 was associated with modifications in the expression of several genes involved in the regulation of protein homeostasis and synaptogenesis.
These results support an implication of the SUMOylation pathway in the mechanisms regulating the intracellular localization of TDP-43, a key protein in the pathophysiology of ALS. An increase in cytoplasmic concentration of TDP-43 is considered toxic. Reducing the nuclear export of TDP-43 by acting on its SUMOylation could have a neuroprotective effect in a context of ALS.

1952: **Cellular stress and alterations in neuronal lipidome in the pathophysiology of ALS.**

Rossi C (*), Romero R (1), Torres P (1), Ayala V (1), Boada J(1) , Pamplona R (1) , Portero-Otín M (1).
(1) Metabolic Pathophysiology Research Group, Department of Experimental Medicine, University of Lleida-IRBLleida, Lleida, Spain.

ALS pathogenesis involves autophagic alterations due to poor protein folding with changes in cellular lipidomes. Lipid Droplets (LDs) constitute the intracellular compartments of lipid reserve. It is known about its dynamism, its participation in many cellular processes and its interaction with other cellular compartments. The existence of multiple links between LDs and neurodegeneration has also been demonstrated. To check if the cellular stresses that occur in ALS can determine alterations in the production of lipid reserves in the form of LDs, cells of murine neuroblastoma (Neuro2a) have been treated with 400 mM sorbitol in a time course experiment, inducing osmotic stress with a consistent cytosolic aggregation of TDP-43. Subsequently, cells were subjected to a specific protocol for the visualization of intracellular LDs with the hydrophobic dye Nile Red. Confocal microscopy images were acquired. LDs have been quantified by a biological image analysis software. By means of biochemical analysis by Western Blot we have explored the relationship between LDs synthesis and autophagy treating the cells with sorbitol together with the autophagy inhibitors bafilomicyn and T863. The preliminary results of the study show that the osmotic stress induced by sorbitol is associated with a LDs biosynthesis of LDs when cells are treated during 90 minutes minimum. When co-treated with bafilomycin 50 µM for 3 h, a significant increase in the autophagic rate is observed. LDs synthesis in response to cellular stress could have the important function of providing energy to the mitochondria through the transport of fatty acids into these organelles, where they undergo _-oxidation. The molecular components of the contact points between LDs and mitochondria are currently unknown.

1956: **Pathogenicity of TDP-43 fragments in Amyotrophic Lateral Sclerosis.**

Anna Chami(1)(*), Rudolf Hergesheimer(1), Philippe Corcia(1,3), Christian Andres(1,2), Pierre Martineau(4), Hélène Blasco(1,2), Patrick Vourc'h(1,2).
1 UMR 1253, iBRAIN, Université de Tours, Inserm, Tours, France.
2 CHU de Tours, Service de Biochimie et Biologie Moléculaire, Tours, France.
3 CHU de Tours, Service de Neurologie, Tours, France.

4 IRCM, Montpellier, France; Inserm, U896.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a progressive and irreversible loss of cortical and spinal motor neurons. The formation of protein aggregates in these motor neurons is involved in the pathogenesis of ALS. Transactivation response (TAR) DNA-binding protein (TDP-43) is identified as a major protein in these aggregates. This nuclear protein is delocalized in the cytoplasm in motor neurons in ALS, where it forms insoluble, ubiquitinated and hyperphosphorylated aggregates.
Post-mortem studies in ALS also identified several truncated forms of TDP-43. Fragment generation appears to be involved in aggregate formation and might increase cytotoxicity. The biochemical properties and the functional roles of these TDP-43 fragments on neurodegeneration remain poorly understood. First we listed the 7 cleavage sites responsible for the truncated forms of TDP-43 identified in post-mortem brains of ALS patients. Interestingly we identified in an ALS patient a new mutation in one of these sites position, p.N291, which is cleaved by an Asparaginyl EndoPeptidase (AEP). The cleavage of TDP-43 at each of the 7 sites resulting in two fragments (N-and C-terminal regions), we cloned the 14 TDP-43 fragments into plasmids. The consequence of their expression on protein aggregation, neurite length and cell viability are studied in motor neuronal cell lines (NSC-34) and in primary cultures of motor neurons. In order to study the pathogenicity of these truncated forms of TDP-43 in relation with aggregation, we searched for intrabodies (ScFv) able to bind TDP-43 protein and to prevent protein aggregation. This phage display protocol was done in partnership with the IRCM.

The aim of this study is to provide a better understanding of the role of TDP-43 fragments in the pathophysiology of ALS, and to open new therapeutic perspectives. This research program is part of an ARD2020 program funded by the Centre Val-de-Loire Region and supported by the Labex MabImprove.

1959: **Secretion of neurotoxic vesicles by muscle cells of ALS patients**.

Stephanie Duguez (1,‡), Laura Le Gall (1,2), William J Duddy(1*,‡), Cecile Martinat (3*), Sylvain Roquevière (3), Mariot Virginie (4,‡), Jeanne Lainé (2), Udayageetha Vijayakumar (1), Susan Knoblach (5), Cedric Raoul (6), Olivier Lucas (6), TRANE study group, Giorgia Querin (7,8), Julie Dumonceaux (4,‡), Gillian Butler Browne (2), Jose-Luis Gonzales De Aguilar (9), Pierre Francois Pradat (1,7,8).
1- Northern Ireland Center for Stratified Medicine, Biomedical Sciences Research Institute, Londonderry, UK.
2- Myologie Centre de Recherche, Université Sorbonne, UMRS 974 UPMC, INSERM, FRE 3617 CNRS, AIM, Paris, France.
3- I-Stem, INSERM/UEVE UMR 861, I-STEM, AFM, Evry, France.
4- NIHR Biomedical Research Centre, University College London, Great Ormond Street Institute of Child Health and Great Ormond Street Hospital NHS Trust, London, UK.
5- George Washington University, Genetic Medicine, Children's National Medical Center, Washington, US.
6- INSERM U1051-Institut des Neurosciences de Montpellier - Déficits sensoriels et moteurs, Montpellier, France.
7- Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France.
8- APHP, Département de Neurologie, Hôpital Pitié-Salpêtrière, Centre référent SLA, Paris, France.
9- Mécanismes Centraux et Périphériques de la Neurodégénérescence, Université de Strasbourg, INSERM U1118, Strasbourg, France.
‡: previous address: Myologie Centre de Recherche, Université Sorbonne, UMRS 974 UPMC, INSERM, FRE 3617 CNRS, AIM, Paris, France.

Despite the discovery of many genetic risk factors, the cause of the motor neuron death that drives terminal pathology in Amyotrophic Lateral Sclerosis (ALS) remains unknown. We report that the skeletal muscle of ALS patients secretes exosomal vesicles that are

specifically toxic to motor neurons. This could not be attributed to a trivial down-stream consequence of muscle denervation. In a study of muscle biopsies and biopsy-derived denervation-naïve differentiated muscle stem cells (myotubes) from 66 human subjects, including healthy and disease controls, ALS myotubes had a consistent signature of disrupted exosome biogenesis and RNA-processing, and their exosomes induced shortened, less branched, neurites, greater death, and disrupted localization of RNA and RNA-processing proteins, in motor neurons. Toxicity was dependent on presence of the FUS protein, which is highly expressed in recipient motor neurons.

1968: **Melatonin-producing pineal gland affected by dipeptide repeat protein pathology.**
Lieselot Dedeene* (1,2,3,4), Koen Poesen# (1,3), Philip Van Damme# (4,5), Dietmar Rudolf Thal# (2,6).
#These authors contributed equally to this work.
1. Department of Neurosciences, Laboratory for Molecular Neurobiomarker Research, KU Leuven (University of Leuven), Leuven Brain Institute (LBI), Leuven, Belgium.
2. Department of Neurosciences, Laboratory for Neuropathology, KU Leuven (University of Leuven), Leuven Brain Institute (LBI), Leuven, Belgium.
3. Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium.
4. Department of Neurosciences, Laboratory for Neurobiology, KU Leuven (University of Leuven) and Center for Brain & Disease Research, VIB, Leuven Brain Institute (LBI), Leuven, Belgium.
5. Department of Neurology, University Hospitals Leuven, Leuven, Belgium.
6. Department of Pathology, University Hospitals Leuven, Leuven, Belgium.

The (G4C2)-hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (C9orf72) is the most common ALS mutation, accounting for half of the familial ALS cases and 10% of the sporadic ALS cases. This repeat expansion also underlies approximately a quarter of the familial frontotemporal lobar degeneration cases. Dipeptide repeat proteins (DPRs) expressed from unconventional repeat-associated non-ATG (RAN) translation of the (G4C2)-hexanucleotide repeat expansion, exhibit distinct types of inclusions. These DPR pathological lesions are mostly restricted to neurons in the central nervous system (CNS), while transactive response DNA-binding protein 43 kDa (TDP-43) pathology extends to glial cells. However, intranuclear DPR inclusions in ependymal cells of the central canal of the spinal cord and in (sub)ependymal cells lining the lateral ventricle were reported. Besides, nuclear inclusions in the Sertoli cells of the testes were observed, whereas other peripheral tissues were shown to be unaffected. In this study, we observed, by immunohistochemical analysis of five C9orf72 repeat carriers, abundant DPR pathology in the melatonin-producing neuroendocrine brain structure, the pineal gland. The relative abundance of DPR pathology was the same as in other CNS regions (poly(GA) > poly(GP)). TDP-43 pathology was not observed. In contrast, the magnocellular cells of the vasopressin-producing supraoptic nucleus were virtually free of DPR pathology. The higher susceptibility of the pineal gland for DPR pathology remains unexplained. Some cases of rapid eye movement sleep behavior disorder (RBD) were reported to carry the C9orf72 repeat expansion, suggesting a role of the C9orf72 repeat expansion in RBD (Daoud et al, 2014). Therefore, the DPR pathological lesions in the melatonin-producing pineal gland could represent a neuropathological correlative for sleep behavior problems in C9orf72 carriers and for similar sleep abnormalities in ALS patients (Lo Coco et al, 2017; Arnulf et al, 2000). In conclusion, the cells of the pineal gland are affected by DPR pathology. The pathogenic role of DPR inclusions in the pineal gland remains unclear, although it could connect neuropathological findings to sleep behavior problems in ALS patients. We plan to extend the study to other neurosecretory cells.
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1978: **Dynactin1 loss-of-function leads to neuromuscular abnormalities without axonal transport deficits.**

Valérie Bercier*(1,2), Jeffrey Hubbard(3), Kevin Fidelin(3), Karine Duroure(1), Thomas Auer(1), Céline Revenu(1), Claire Wyart(3), Filippo Del Bene(1).
1. Institut Curie-Research Center, Developmental Biology , Paris, France
2. VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium
3. Brain and Spinal Cord Institute, Paris, France.

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, which is mainly sporadic in nature. This progressive pathology has an estimated incidence of 1:1000 and generally leads to death within 2-5 years of diagnosis due to muscle wasting and severe motor neuron loss. Over the last years, mutations have been identified in both sporadic and familial ALS patients, interfering with the function of many genes, including DCTN1, which encodes for a subunit of the motor protein complex component dynactin. The dynactin complex serves as an adaptor for the dynein motor complex, responsible for retrograde axonal transport, and it is believed to regulate dynein activity and the binding capacity for cargos. Interestingly, axonal transport deficits have been reported in various neurodegenerative diseases owing to the fact that neurons are highly polarized cells that depend on active axonal transport for growth, establishment and maintenance of synapses.
In order to determine how retrograde axonal transport is involved in the pathogenesis of ALS, we have characterized a mutant zebrafish line for dctn1 with regard to axonal development of primary motor neurons, formation and stability of the neuromuscular junction (NMJ) and the behavioral phenotype produced in embryos.
Fast axonal transport was quantified in primary motor neurons using the GAL/UAS bipartite system and fusion protein tracking in vivo by confocal timelapse microscopy. We have investigated the transport dynamics of cargos such as endosomes, mitochondria, synaptic vesicles and neurotrophic receptors in the motor neurons of wild-type versus dctn1 mutant embryos in vivo, and over time. As dynactin was reported to be essential to synapse stability, we have also examined the formation and maintenance of the NMJ with techniques such as immunohistochemistry, EM, GCaMP calcium imaging, and muscle-motor neuron paired recordings. We found that loss of dctn1 function leads to synapse instability, functional abnormalities at the NMJ, growth defects and impaired locomotion without affecting axonal transport or the regulation of the cytoskeleton. These results suggest that the contribution of Dynactin1 to ALS pathogenesis is mediated by other mechanisms that was previously proposed.

1998: **Investigation of molecular pathological hallmarks and therapeutic strategies in C9orf72 human lines.**

Fabio Biella* (1), Michela Taiana (1), Margherita Bersani (1), Monica Nizzardo (1,2), Mafalda Rizzuti (2), Brunno Rocha Levone (3), Nereo Bresolin (1,2), Giacomo Pietro Comi (1,2), Silvia Barabino (3), Stefania Corti (1,2).
1 Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), Neurology Unit, University of Milan, Milan, Italy.
2 IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
3 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy.

The 40% of familial Amyotrophic Lateral Sclerosis (ALS) cases and 5%-10% of sporadic ALS can be attributed to the hexanucleotide repeat expansion in the locus C9orf72. This mutation likely impairs different key molecular mechanisms giving rise to the complex etiology of this disease, even if the most of them still remain to be clarified. Patient-derived induced pluripotent stem cells (iPSCs) can be a useful tool to model this disease and its relevant phenotypes.

In our Lab, among the pathogenic features analyzed in C9orf72 iPSCs and derived motor neurons (MNs), we found the alteration of DNA damage response, the increase in DNA damage as well as the accumulation of DNA damage-inducing structures called R-loops. We also confirmed the presence of C9orf72 expanded transcripts RNA foci, structures known to sequester RNA-binding protein. Moreover, we found Pur-_, a molecular actor believed to play a role in R-loops homeostasis, together with RanGAP, a protein involved in nuclear trafficking, to be accumulated and mislocalized. From a different pathogenic prospective, by a high-throughtput gene card assay, we identified and validated a subset of miRNA deregulated in C9orf72 lines compared to healthy controls. The investigation of the downstream target genes and their roles in the pathology is currently ongoing.
Beside the research on ALS pathological mechanisms, we designed antisense oligonucleotides with a particular chemistry, morpholino (MO) oligomers, to target C9orf72 as a therapeutic strategy. Preliminary results on MO efficacy on pathological hallmarks observed are promising.
In addition, we are exploiting the CRISPRCas9-mediated gene editing technique in order to produce isogenic mutation-corrected iPSC lines as tool for basic research and as a platform to test the feasibility of a gene editing-based therapeutic approach.
Overall we demonstrated that patient specific iPSC-derived lines are a valuable tool to deepen the knowledge of C9ORF72 pathogenic mechanisms and to validate new therapeutic strategies.

2001: **FUS localization is changed from the postsynapse to the presynapse during motoneuron development and accumulates in human ALS synapses.**

Dhruva Deshpande (2), Julia Higelin (1), Michael Schoen (1), Thomas Vomhof (2), Tobias M. Boeckers (1), Jens Michaelis (2) and Maria Demestre (1)*.
1 Institute for Anatomy and Cell Biology, Ulm University, Ulm, Germany.
2 Institute of Biophysics, Ulm University, Ulm, Germany.

In motoneurons an abnormal cytoplasmic accumulation of the mutated DNA/RNA binding protein fused in sarcoma (FUS) is observed, in some amyotrophic lateral sclerosis (ALS) cases. FUS is mainly confined to the nucleus, but in neurons it shows an additional somatodendritic localization. Maturation based changes in the subcellular localisation of FUS at motoneuron synapses were analysed in primary motoneurons. To distinguish between pre- and post-synaptic compartments super-resolution microscopy was used. In early stages of synaptic development FUS was post-synaptic, while in mature synapses FUS changed to the pre-synapse. Further, in human neurons motoneurons derived from induced pluripotent stem cells FUS was post-synaptic but in ALS FUS patient motoneurons, FUS and other synaptic markers aberrantly accumulated in synapses. Having demonstrated dynamic changes in the FUS localisation during synaptogenesis, we propose a role of synaptic FUS in dendritic and axonal compartments, probably affected by the accumulation mutant FUS in ALS.

2005: **The ER chaperone Sigma receptor 1 (SigR1) in RNA binding protein homeostasis in ALS .**

Haihong Guo (1)#, Priyanka Tripathi (1)#, Lissane van `t Sant (1), Alfred Yamoah (1), Istvan Katona (1), Jasper Anink (2), Dirk Troost (2), Eleonora Aronica (2), Joachim Weis (1)# , Anand Goswami (1)#*.
# Equal contribution
1 Institute of Neuropathology, RWTH Aachen University Medical School, Pauwelsstr. 30, 52074 Aachen, Germany

2 Amsterdam UMC, University of Amsterdam, Department of (Neuro) Pathology, Amsterdam Neuroscience, Amsterdam, The Netherlands.

Mutant / misfolded proteins and their aggregates primarily affect two vital cellular machineries in neurons, RNA metabolism and protein quality control. Crosstalk among these processes initiates pathological cascades and potentiates further accumulations of RBPs and neurodegeneration as often seen in ALS. Mutations in the endoplasmic reticulum (ER) chaperone Sigma receptor 1 (SigR1) have been reported to cause juvenile ALS, ALS-FTD and autosomal recessive distal hereditary motor neuropathy (dHMN). Lack of SigR1 exacerbates ALS progression in G93A-SOD1 mice. Similarly, MN degeneration, loss of neuromuscular junctions, neurogenic muscular atrophy as well as decreased rota-rod performance and life span has been reported in SigR1 knockout mice. We previously described abnormal modification as well as altered localization of SigR1 protein in the human sporadic (s) ALS patient spinal cord. Consistent with this we showed that depletion of SigR1 leads to ER proliferation / expansion, autophagy defects and ER stress-mediated cell death. E102Q-SigR1-mediated ALS pathogenesis comprises a vicious circle of altered ER structure, autophagy alterations and defective RBP homeostasis fed also by gain-of-function mechanisms. These results are indicative of SigR1 protein regulating ER size, selective autophagy and RBP homeostasis. Still it is unclear how these pathways are interconnected and pathologically orchestrated in ALS to induce neurodegeneration. In the present study we observed that elevated levels of SigR1 are associated with reduction of pTDP43 and FUS aggregates in the lumbar spinal cord _-MNs of ALS patients. Consistent with this observation, loss of SigR1 leads to autophagy defects and to the cytoplasmic mislocalization of RBPs including FUS, TDP-43 and Matrin 3 in cell culture models. Lumbar spinal cord _-MNs of SigR1 knockout mice also showed signs of autophagy dysfunction as well as cytoplasmic accumulation of pTDP-43. In summary our results supports the notion that autophagy and RBP homeostasis are tightly regulated by SigR1 and imbalances in these processes contributes to neurodegeneration in ALS.

2006: **Transcriptomic analysis of C9orf72 iPSC-derived motor neurons identifies a dysregulation in vesicle transport pathways.**

Ana Candalija*(1), Jakub Scaber (1), Ruxandra Dafinca (1), Viola Volpato (2), Caleb Webber (2), Elke Braems (3), Ludo van den Bosch (3), Georg Haase (4), Kevin Talbot (1).
(1) Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom.
(2) Department of Physiology, Anatomy and Genetics, University of Oxford and Dementia Research Institute, Cardiff University, United Kingdom.
(3) VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium.
(4) Institute of Systems Neuroscience, INSERM & Aix-Marseille University, Marseille, France.

Background: Induced pluripotent stem cell-derived motor neuron (iPSC-MN) cultures reproduce in vitro some of the typical features of C9-related ALS/FTD, such as the presence of RNA foci and dipeptide repeats (DPRs), activation of apoptosis, calcium dysregulation, stress granule formation and protein aggregation. The use of isogenic CRISPR/Cas9 edited controls and FAC sorting produces pure iPSC-MNs, minimising the intrinsic variability of these cultures, with the potential to unmask important biological alterations in the transcriptomic profile of C9 iPSCs-MNs.
Objectives: The aim of this project is to identify differentially expressed genes in pure iPSC-MN cultures from C9orf72 patients compared to isogenic C9-corrected lines and age-matched healthy controls, and to determine whether these are effectors of G4C2-triggered neurodegeneration.
Results: We performed RNA sequencing on fluorescence-activated cell sorted (FACS) iPSC-MNs carrying the C9orf72 expansion and compared their transcriptomic profile to isogenic

C9-CRISPR/Cas9 corrected lines. The study included two iPSC clones from a C9orf72 patient, three isogenic iPSC C9-CRISPR/Cas9 corrected clones, and two healthy controls. Phenotypic linkage network analysis and GO annotation identified synaptic transmission and vesicle docking processes as dysregulated in C9-MN. The top ranked differentially expressed genes in these categories were further validated in iPSC-MN at mRNA level by using qRT-PCR, and at protein level by western blot and immunostaining. Synaptotagmin 11 (Syt11), a protein involved in endocytic and exocytic pathways, is overexpressed in C9-MNs. To evaluate its functional role, we knocked-down or overexpressed it in a zebrafish model expressing G4C2 repeats, and results indicates a protective effect of Syt11 on axonal length.
Conclusions: Further work will include the detailed study of vesicle trafficking pathways in C9-MNs and the effect of overexpression/knock-down of the key targets identified in this study. iPSC-MN cultures are an effective model to investigate early ALS-related neurodegeneration and the selected effector pathways in this study might be candidates as 'druggable' targets for future neuroprotective strategies.
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2008 **The GRP78 co-chaperon SIL1 in ALS.**

Alfred Yamoah (1), Haihong Guo (1), Priyanka Tripathi (1), Istvan Katona (1), Jasper Anink (2), Dirk Troost (2), Eleonora Aronica (2), Andreas Roos (3), Anand Goswami (1), Joachim Weis (1)*.
1) Institute of Neuropathology, RWTH Aachen University Medical School, Aachen, Germany.
2) Department of (Neuro) Pathology, Amsterdam UMC, Amsterdam Neuroscience, University of Amsterdam, Amsterdam, The Netherlands.
3) Leibniz Institute of Analytical Science – ISAS, Dortmund, Germany.

Abnormal accumulations of misfolded proteins that elicit endoplasmic reticulum (ER) stress are pathological hallmarks of amyotrophic lateral sclerosis (ALS). Mutation of the ER co-chaperon SIL1 causes Marinesco-Sjögren syndrome (MSS; cerebellar degeneration, cataracts and vacuolar myopathy). Recent studies by us and others showed that SIL1 mutation leads to specific ER alterations in patients and mice and that SIL1 regulates motor neuron subtype-selective ER stress in SOD1 ALS mice. Using SIL1 knockdown and overexpressing cells we found that SIL1 deficiency leads to severe alterations in autophagy pathways. Moreover, our recent studies on cellular model systems using advanced proteomics confirmed that SIL1 depletion leads to ER and nuclear envelope widening and proliferation, ER stress, activated UPR and ERAD and altered autophagy as well as increased expression of GRP78 and other GRPs. Further recent results indicate that SIL1 and its binding partner GRP78 associate with the Nissl substance of disease-resistant human _-MNs and that SIL1 often co-localizes with abnormal intraneuronal aggregates in human ALS. Taken together, these data suggest that SIL1 protects motor neurons from damage elicited by ER stress, impaired autophagy and abnormal accumulation of misfolded RNA-binding proteins linked to ALS pathogenesis.

2011: **Understanding the interaction between C9ORF72-dipeptide repeats and nucleocytoplasmic transport.**

Joni Vanneste (1,2)*, Mathias De Decker (1,2), Thomas Vercruysse (3), Steven Boeynaems (4), Bob Massant (3), Liesbeth Mercelis (4), Wendy Scheveneels (1,2), Adrian Isaacs (5,6), Dirk Daelemans (3), Ludo Van Den Bosch (1,2).
1. KU Leuven - University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), 3000 Leuven, Belgium
2. VIB, Center for Brain and Disease Research, Laboratory of Neurobiology, 3000 Leuven, Belgium

3. KU Leuven Department of Microbiology and Immunology, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, 3000 Leuven, Belgium
4. Department of Genetics, Stanford University School of Medicine, Stanford, USA.
5. Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK
6. UK Dementia Research Institute at UCL, UCL Institute of Neurology, Queen Square, London, UK.

A hexanucleotide repeat expansion (G4C2) in the C9ORF72 gene is the most common genetic cause known so far of amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD). This repeat expansion, which can extend up to hundreds or thousands of times, gives rise through non-conventional repeat translation to the production of dipeptide repeats (DPRs): poly-GP, poly-GA, poly-GR, poly-PA and poly-PR. We, and others, have previously suggested a link between DPR-induced toxicity and nucleocytoplasmic transport. By using a targeted RNAi-screen in poly-GR expressing flies, we observed that proteins involved in nucleocytoplasmic transport are modifiers of GR pathology, supporting the hypothesis of a potential interaction between DPRs and nucleocytoplasmic transport. However, it is not clear what the exact interaction is between DPRs and nucleocytoplasmic transport. In order to assess whether different DPRs directly disturb nucleocytoplasmic transport, we made use of Hela Kyoto cells endogenously expressing a shuttling NLSSV40-2*mNeonGreen-NESpki construct, which is mainly localized in the cytoplasm in steady state conditions. DPRs where either added to the cell cultures (PR20, GR20) as peptides or expressed making use of lentiviral vectors (GA100, PA100, GR100, PR100). By blocking export we can thereafter measure active nuclear import of the mNeonGreen construct in time. We could conclude that these DPRs do not induce import defects. This suggests that a direct induced transport defect may not underlie the observed nucleocytoplasmic transport-DPR toxicity interaction. However, there is need for further research, in which we are focusing on iPSC-derived MNs expressing these DPR constructs, to understand the role of nucleocytoplasmic transport in C9orf72-ALS.

2030: **The molecular pathogenesis of SOD1-linked ALS is promoted by low oxygen tension.**

Isil Keskin (1)*, Elin Forsgren (2), Manuela Lehmann (2), Peter M. Andersen (2), Thomas Brännström (1), Dale J. Lange (3), Matthis Synofzik (4,5), Ulrika Nordström (2), Per Zetterström (6), Stefan L. Marklund (6) and Jonathan D. Gilthorpe (2).
1) Department of Medical Biosciences Pathology, Umeå University, Umeå, Sweden.
2) Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden.
3) Department of Neurology, Hospital for Special Surgery and Weill Cornell Medical Center, New York, USA.
4) Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany.
5) German Research Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany.
6) Department of Medical Biosciences, Clinical Chemistry, Umeå University, Umeå, Sweden.

Background and aim: Mutations in superoxide dismutase 1 (SOD1) are a well-known cause of amyotrophic lateral sclerosis (ALS). Disease pathogenesis is linked to destabilization, disorder and aggregation of the SOD1 protein. However, the non-genetic factors that promote the onset or progression of SOD1-mediated ALS are not understood. We hypothesized that age, as the principal non-hereditary risk factor for ALS, together with other suggested risk factors including smoking, strenuous physical activity and ischemia etc. could be linked to disease pathogenesis by a common mechanism involving reduced vascular perfusion, leading to transient or chronic local CNS hypoxia.
Mainly located to the reducing cytosol, mature SOD1 contains an oxidized disulfide bond that is important for its stability. Since oxygen is required for formation of the bond, we

reasoned that low oxygen tension might be a risk factor for the pathological changes associated with ALS development.
Methods and results: By combining biochemical approaches in an extensive range of genetically distinct patient-derived cell lines, we show that the disulfide bond is an Achilles heel of the SOD1 protein. Culture of patient-derived fibroblasts, astrocytes, and induced pluripotent stem cell-derived mixed motor neuron and astrocyte cultures (MNACs) under low oxygen tensions caused reductive bond cleavage and increases in disordered SOD1. The effects were greatest in cells derived from patients carrying ALS-linked mutations in SOD1. However, significant increases also occurred in wild-type SOD1 in cultures derived from non-disease controls, and patients carrying mutations in other common ALS-linked genes. Compared to fibroblasts, MNACs showed far greater increases in SOD1 disorder and even aggregation of mutant SOD1s, in line with the vulnerability of the motor system to SOD1-mediated neurotoxicity.
Conclusions: Our results show for that oxygen tension is a principal determinant of SOD1 stability in human patient-derived cells. Furthermore, we provide a mechanism by which non-genetic risk factors for ALS, such as aging and other conditions causing reduced vascular perfusion, could promote disease initiation and progression.

2033: **Intercellular miscommunication in the brain and periphery: characterization of extracellular vesicle in Amyotrophic Lateral Sclerosis.**

Deborah Ferrara(*1), Laura Pasetto(2), Alessandro Corbelli(2), Fabio Fiordaliso(2), Andrea Calvo(3), Adriano Chiò(3), Massimo Corbo(4), Christian Lunetta(5), Gabriele Mora(6), Vito D'Agostino(1), Valentina Bonetto(2), Manuela Basso(1), but in the Abstract book you wrote Andrea Calvo(3), Adriano Chiò(3), Massimo Corbo(4), Christian Lunetta(5), Gabriele Mora(6), Vito D'Agostino(1), Valentina Bonetto(2), Manuela Basso(1).
(1) Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy.
(2) IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.
(3) CRESLA, department of neuroscience Rita Levi Montalcini , Università degli Studi di Torino, Torino (Italy).
(4) Casa di Cura del Policlinico, Milano (Italy).
(5) NeuroMuscular Ominicentre, Fondazione Serena Onlus, Milano (Italy).
(6) Fondazione Salvatore Maugeri Clinica del Lavoro e della Riabilitazione-IRCCS, Milano (Italy).

Amyotrophic Lateral Sclerosis (ALS) is a progressive and devastating motor neuron disease that affect upper and lower motor neurons. Among several factors, the progression of the disease is mediated by altered intercellular communication in the spinal cord between neurons and glial cells. One of the possible way in which the cell-to-cell communication occurs is through extracellular vesicles (EVs) able to transport proteins, lipids and nucleotides from one cell to the other. Previously, we proved that EVs released from ALS mutant SOD-1 astrocytes selectively induced death in wild type motor neuron, suggesting EVs as mediators of toxicity. Our hypothesis is that EVs might constitute a mechanism for local and systemic intercellular transfer of proteins and genetic information in the form of RNA, responsible for driving disease progression and contribute to motor neuron degeneration. To characterize EVs in ALS, we are using a novel methodology, called NBI, a beads-based methodology, allowing rapid and efficient purification of EVs. By this approach, we are able to recover not only a higher and purer amount of astrocyte-derived EVs, but also to preserve the integrity and the stability of vesicles to analyze their cargo content. Our preliminary results suggest that EVs derived from a transgenic mouse model of ALS overexpressing mutant TDP-43 (Q331K) transmit toxicity to wild type neurons and are different in number and size compared with control mice, suggesting that in ALS, EV biogenesis is altered. To test whether the defect is system or CNS-specific, we investigated the similarities between astrocyte-derived EVs and plasma EVs obtained from two different

ALS mouse models and human patients. Interestingly, the change in EV amount and size was confirmed in all the samples analyzed. To detect whether the neuron and glia-derived EVs are present in the plasma, we set up a new methodology to define the relative percentage of CNS-derived EVs in periphery. Concluding, ALS EVs present different biochemical properties compared to controls, suggesting that their characterization could be of use for biomarker discovery and mechanistic investigations.

2034: **The emerging role of variants in the 3'UTR of FUS among genetic factors underlying ALS.**

Giuseppe Marangi (1)*, Serena Lattante (1), Paolo Niccolò Doronzio (1), Amelia Conte (2), Giulia Bisogni (2), Daniela Bernardo (2), Agata Katia Patanella (2), Marcella Zollino (1), Mario Sabatelli (2).
1) Institute of Genomic Medicine, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy.
2) Centro Clinico Nemo, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy.

Exonic untranslated regions (UTRs) are usually neglected in the search for pathogenic mutations. However, causative variants in both 5' and 3' UTRs have been identified for several human disorders and recent studies suggest that these regulatory regions may also be involved in the pathogenesis of amyotrophic lateral sclerosis. Specifically, variations in the 3'UTR of FUS have been consistently associated with ALS, in principle by altering FUS transcript levels.
In the present study, we investigated for the presence of rare FUS 3'UTR variants in a group of 535 consecutive ALS patients, by using next-generation targeted sequencing of a panel of 36 genes. This approach allowed us to analyse the whole UTR region and to identify the co-occurrence of variants in other ALS genes. Only variants with a minor allele frequency (MAF) lower than 1% in the European population (NFE, gnomAD database) were considered. A set of 56 different variants in the 3'UTR of FUS were detected in 121/535 (22.6%) patients compared to 16/196 (8.1%) control subjects (pT and c.*41G>A have been consistently identified as polymorphisms, we excluded them from further analyses. The frequency of the remaining variants were 18.9% (101/535) in the ALS patients and 4.08% (4/196) in controls (OR 5.46; CI 2.6-11.4 pT was detected in 6% of our patients while was not present in our controls and is reported at a frequency of 0.01% in gnomAD NFE. In ten patients and one control we identified the co-occurrence of variants c.*491T>G, c.*1144_*1145delAC, c.*1803C>T, c.*2486G>A, c.*2794G>C.
Among the 101 patients with variants in the 3'UTR of FUS, 49 (48.5%) had additional variants in one or more of the analysed ALS-related genes.
Our results indicate that variants in 3'UTR of FUS could be included among the genetic factors associated with an increased risk of developing ALS, being present in about 20 % of patients. Altered FUS autoregulation, caused by these variants, can trigger gain-of-function toxicity via altered autophagy-lysosome pathway and RNA metabolism function, mediated toxicity. The observation that a significant number of patients with variants in the 3'UTR of FUS carry additional variants in other ALS-related genes can be explained in the view of a possible oligogenic model for ALS.

2038: **mRNA export factor GANP links to TDP-43 homeostasis.**

Rosa Woldegebriel (*)(1,2), Matthew White (2), Emil Ylikallio (1,3), Laura Mäenpää (1), Carsten Bonnemann (4), Sandra Donkervoort (4), Diana Bharucha-Goebel (4), Maie Walsh (5), Zornitza Stark (5), Marie-José van den Boogaard (6), Pirjo Isohanni (1,7), Tuula Lönnqvist (7), Andrew Bassett (8), Jemeen Sreedharan (2), Henna Tyynismaa (1,9).
1 Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland.

2 Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.
3 Clinical Neurosciences, Neurology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.
4 Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA.
5 Murdoch Children's Research Institute, Victoria, Australia.
6 Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands.
7 Department of Child Neurology, Children's Hospital and Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.
8 Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom.
9 Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland.

Alterations in nucleocytoplasmic transport underlie both axon degeneration and amyotrophic lateral sclerosis (ALS). In a genetic screen of fly motor neurons, a loss of function mutation in xmas-2 (human ortholog MCM3AP, encoding germinal center associated nuclear protein, GANP) was found to suppress toxicity due to TAR DNA-binding protein 43 (TDP-43) overexpression, central in the pathogenesis of ALS. Furthermore, we recently described patients from five families with early-onset neuropathy and intellectual disability that had mutations in MCM3AP, thereby linking GANP to human neurodegeneration. We are currently studying the interaction between TDP-43 and GANP and how their relationship contributes to pathophysiological changes in neurodegeneration.
GANP is a scaffold protein in the TRanscription-EXport-2 (TREX-2) complex that interacts with the nuclear pore. It plays a key role in exporting selective mature mRNAs and stress-related transcripts from the nucleus. The protein has an mRNA-binding Sac3 (suppressor of actin) domain, where homozygous mutations cluster. Compound heterozygous mutations outside of the domain cause a loss of GANP protein. Earlier onset and a severe disease course are associated with the compound heterozygous mutations, whereas a benign disease with the homozygous Sac3 mutations.
We analyzed MCM3AP patient fibroblasts along with matched controls. Mutations of MCM3AP outside the Sac3 domain caused a reduction in GANP expression in the nuclear envelope. Transcriptomics of fibroblasts using RNA sequencing confirmed MCM3AP reduction in 5 different patient lines and identified common differentially-expressed genes such as Netrin 4 (NTN4) and Small Nucleolar RNA Host Gene 5 (SNHG5). Additionally, in vitro siRNA knockdown of GANP reduced TDP-43 protein levels, confirming a mechanistic link between GANP and TDP-43.
Our results suggest that defective mRNA binding through the conserved Sac3-domain as well as a severe reduction in GANP protein compromise mRNA export and reduce expression of TDP-43, contributing to neurodegeneration.
We are now utilizing human wild-type induced pluripotent stem cells (iPSCs) generated using CRISPR/Cas9 and differentiated neurons to investigate the role of GANP in health and disease. We identified that GANP is expressed in both iPSCs and cultured neurons. Using derived sensory and motor neurons we will model GANP mutations and TDP-43 expression to dissect how these two proteins link to disease.

2050: **The impact of Ryanodine receptor and Inositol-1,4,5 receptor on organelle function in IPSC-derived ALS motor neurons.**

Benjamin Vlad* (1), Vedrana Tadic (1), Nayana Gaur (1), Beatrice Stubendorff (1), Otto W Witte (1), Andreas Hermann (2), Julian Grosskreutz (1).
(1) Hans Berger Department of Neurology, Jena University Hospital, Jena, Germany.
(2) Department of Neurology, University Hospital Dresden, Dresden, Germany.

Background: Organelle dysfunction in motor neurons appears to play a major role in ALS pathophysiology. Both the Ryanodine receptor (RyR) and Inositol-1,4,5 receptor (IP3R) are crucial components of the Endoplasmic Reticulum-Mitochondria-Calcium-Cycle (ERMCC), as they induce calcium efflux from the ER. As ER calcium depletion is a central aspect of the complex pathogenesis in ALS, disturbances in expression and function of RyR and IP3R are likely to be involved in the perturbation of the ERMCC.
Objectives: The individual roles of the RyR and IP3R in organelle dysfunction in ALS will be explored to better understand the potential for therapeutic modulation. To achieve this, we will (i) investigate receptor expression and localization and (ii) whether targeted modulation can rescue MNs from kainite-induced excitotoxicity. Furthermore, we aim to analyze and compare (iii) neurite length and (iv) branch points of SOD1R115G mutation-carrying and control MNs.
Methods: Induced pluripotent stem cells-derived MNs from a) patients carrying the ALS SOD1R115G mutation and b) healthy controls were used for immunofluorescent staining and live-cell imaging. Neurite length and branch points were compared under different conditions over 18 days. Neuronal cell survival was assessed after RyR- and IP3R receptor treatment and kainate-induced excitotoxicity.
Results: Pilot experiments indicate that RyR manipulation increases MN survival following kainate-induced excitotoxicity significantly. Further, no significant differences in either RyR or IP3R cellular distribution were apparent between SOD1R115G and control MNs. Nevertheless, additional experiments will be performed to confirm this. . SOD1R115G MNs showed reduced neurite length and branch point counts relative to control MNs. Interestingly, SOD1R115G MNs appeared to be more resilient to media-starvation, indicating potential compensatory mechanisms at play. Additional experiments to further dissect this are underway.
Acknowledgment: This research is supported by a BMBF (the Bundesministerium für Bildung und Forschung) grant PYRAMID in the framework of the ERANET E-RARE program (http://www.e-rare.eu)and the Dt. Gesellschaft für Muskelkranke (DGM).

2064: **Capturing ALS: LCM-Seq for single-cell spatial transcriptomic profiling of human spinal motor neurons.**
Christoph Schweingruber*(1), Jik Nijssen(1), Julio Aguila Benitez(1), Gill Pollmeier(1), Nigel Kee(1), Eva Hedlund(1).
(1) Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which somatic motor neurons in the cortex, brainstem, and spinal cord progressively degenerate, leading to spasticity, muscle atrophy, and paralysis. Although functional characteristics of spinal motor neurons in man are well established , their transcriptional signatures and possibly greater diversity in health and ALS remain elusive. The spinal motor neurons are often classified into three groups based on the type of muscle fiber they innervate; namely slow-twitch (S), fast-twitch fatigue-resistant (FR), and fast-twitch fast-fatigable (FF). The FF motor neurons are considered the most vulnerable in ALS, while S are comparably resilient. Here we study individual motor neuron somas in human post mortem spinal cord tissue using laser capture microscopy coupled with mRNA sequencing (LCM-Seq) in order to resolve their transcriptional diversity and further delineate subpopulations that are vulnerable and resilient to ALS. Because LCM-Seq enables both robust and sensitive detection of expressed genes, it permits the precise analysis of cell populations while preserving the spatial information even in scarce and partly degraded human post mortem tissue. We present our preliminary data on the diversity of human spinal motor neurons at single cell resolution in control tissue and show that we can distinguish motor neuron subtypes. Also our analysis establishes reliable markers for the spinal MN populations. We anticipate that this analysis

will further our understanding of the selective and differential vulnerability of spinal motor neurons and help delineate the underlying molecular mechanisms.

2073 : **TDP-43 Regulation of Acetylcholinesterase Splicing and Implications for NMJ structure**.

Maria-Letizia Campanari1, Anca Marian1, Gina Picchiarelli2, Luc Dupuis2, Jose-Luis González De Aguilar2, Sorana Ciura1, and Edor Kabashi1.
(1) Sorbonne Université, Université Pierre et Marie Curie (UPMC), Université de Paris 06, Unité Mixte 75, Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1127, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche 7225 Institut du Cerveau et de la Moelle Épinière (ICM), 75013, Paris, France.
(2) Université de Strasbourg, UMR_S 1118, Strasbourg, France.

One of the earliest and most prevalent pathogenic features in ALS consists in structural and functional defects of the neuromuscular junction (NMJ). The pathophysiological cascade leading to the dismantling of the NMJ, resulting in failure of neuromuscular transmission and paralysis, is not clearly understood.
The RNA and DNA binding protein TDP-43 is considered the pathological signature of ALS, being the principal component of proteinaceous inclusions found in affected tissues in more than 95% of both familial and sporadic ALS cases. TDP-43 can function at multiple levels of mRNA processing, including splicing, transport and nuclear-cytoplasmic shuttling. Recent evidence has implicated widespread dysregulation of RNA splicing as a common feature in ALS patients and in TDP-43 models of the disease. However, the relevance of specific splicing targets to ALS disease processes remains to be defined.
In a transcriptomic dataset obtained from ALS muscle biopsies, we have identified a set of key transcripts involved in NMJ stability which are deregulated early in the course of the disease, including acetylcholinesterase (AChE), its anchor protein ColQ and a the splicing factor SRSF2, previously identified as TDP-43 interactor. Using in vitro and in vivo models we have determined that TDP-43 functionally interacts with splicing factors SRSF1 and 2 to control a key switch of splice variants of the AChE transcript that affects its role at the NMJ. Thus, we have been able to link for the first time the regulation of splicing by TDP-43 with a highly relevant mechanism in ALS that is involved in the maintenance of the NMJ. Our results provide a better understanding of the role of TDP-43 in the NMJ organization and indicate AChE as a contributing factor in the pathology of ALS.

2076: **SIRT2 is overexpressed in NSC34(SOD1-G93A) cells and contributes to the control of reactive oxygen species production**.

Alonso-Munguía G*(1),Rubio-Alarcón M (1),Yunta M (1), Campos Y (1).
Unidad de Patología Mitocondrial. Instituto de Salud Carlos III de Madrid. Spain.

Amyotrophic lateral sclerosis (ALS) is a degenerative disorder characterized by a selective loss of upper motor neurons in the motor cortex and of lower motor neurons in the spinal cord and brainstem, culminating in respiratory insufficiency and patient´s death 3–5 years after diagnosis. ALS is usually sporadic (sALS), but approximately 10% of cases are familial (fALS), being inherited mainly in an autosomal dominant manner. Among them, 20 % are due to mutations in the gene that codify for the protein Cu2+/Zn2+ superoxide dismutase (SOD1). A characteristic of this pathology is the degeneration of the mitochondria associated to the increment of reactive oxygen species (ROS).
Sirtuin-2 (SIRT2) is one of the seven mammalian sirtuins, a family of proteins NAD+-dependent histone deacetylases (SIRT1-7) with homology to the yeast silent information regulator 2 (Sir2). SIRT2 is the most abundant sirtuin in the brain, located preferentially in the cytoplasm of neurites and growth cones. SIRT2 accumulates in an age-dependent

manner in mouse brain and spinal cord and it has been observed that SIRT2 knock-down enhances microtubule acetylation and resistance to axonal degeneration. Moreover, inhibition of its function is thought to improve the course of neurodegenerative disorders such as Parkinson's or Huntington's disease.
In this work we studied ROS production and cell viability in the ALS cellular model NSC-34 (wild-type WT and SOD1G93A). SIRT2 and _-tubulin acetylation levels were determined by western blot. To establish if the inhibition of SIRT2 affected ROS generation or acetylation of _-tubulin we used the specific SIRT2 inhibitor AK1.
Reactive oxygen species and cell mortality were increased in the SOD1G93A cells compared to controls. Western blot analysis showed that there was a significant increase in the expression of SIRT2 in the NSC-34(SOD1G93A) clones. This event may be related with the substantial reduction that we observed in the acetylation of the SIRT2 protein target _-tubulin. However, AK1 treatment induced a significant an increase of ROS and reduction of cell viability with no evident modification of _-tubulin acetylation.
This work was supported by Spanish Foundation for the research in amyotrophic lateral sclerosis (FUNDELA).

2077: **FUS ALS-causative mutations impact FUS autoregulation and the processing of RNA-binding proteins through intron retention.**

Jack Humphrey(1), Nicol Birsa(1), Carmelo Milioto(2), David Robaldo(2), Matthew Bentham(1), Seth Jarvis(1,3), Cristian Bodo(1), Maria Giovanna Garone(4), Anny Devoy(1,2,5), Alessandro Rosa(4,6), Irene Bozzoni(4,6), Elizabeth Fisher(1), Marc-David Ruepp(2,5), Giampietro Schiavo(1,7), Adrian Isaacs(1,2), Vincent Plagnol(3), Pietro Fratta(1) .
1. UCL Queen Square Institute of Neurology, University College London, London , UK.
2. UK Dementia Research Institute, London, UK
3. UCL Genetics Institute, University College London, London , UK.
4. Sapienza University of Rome, Rome, IT.
5. Maurice Wohl Clinical Neuroscience Institute, King's College London, London, UK.
6. Center for Life Nano Science, Istituto Italiano di Tecnologia, Rome, IT.
7. Discoveries Centre for Regenerative and Precision Medicine, University College London Campus, London, UK.

Mutations in the RNA binding protein FUS are a cause of amyotrophic lateral sclerosis (ALS), the devastating neurodegenerative disease in which the loss of motor neurons induces progressive weakness and death from respiratory failure, typically only 3-5 years after onset. FUS has a role in numerous aspects of RNA processing, including splicing. However, the impact of ALS-causative mutations on splicing has not been fully characterized. Most disease models have been based on FUS overexpression, which in itself alters its RNA processing functions, making it hard to disentangle the effect deriving from overexpression and mutations on splicing. To overcome this, we and others have recently created knock-in models, and have generated high depth RNA-sequencing data on FUS mutants in parallel to FUS knockout. We combined three independent datasets with a joint modelling approach, allowing us to compare the mutation-induced changes to genuine loss of function. We find that FUS ALS-mutations induce a widespread loss of function on expression and splicing, with a preferential effect on RNA binding proteins. Mutant FUS induces intron retention changes through RNA binding, and we identify an intron retention event in FUS itself that is associated with its autoregulation. Altered FUS regulation has been linked to disease, and intriguingly, we find FUS autoregulation to be altered not only by FUS mutations, but also in other genetic forms of ALS, including those caused by TDP-43, VCP and SOD1 mutations, supporting the concept that multiple ALS genes interact in a regulatory network.

2078: **Loss of optineurin function in microglia leads to a disbalance in pro- and anti-inflammatory factors regulated by IFN-beta.**

Andrea Markovinovic (1), Tereza Ljutic (1), Louis-Charles Béland (2), Nikolina Prtanjaca (1), Marin Dominovic (1), Ivana Munitic (1*).
(1) Laboratory for Molecular Immunology, Department of Biotechnology, University of Rijeka, Rijeka, Croatia.
(2) CERVO Research Centre, Laval University, Quebec City, Canada.

Mutations in optineurin have been found in amyotrophic lateral sclerosis (ALS) patients. Optineurin is a ubiquitin-binding adaptor protein proposed to regulate several processes including inflammatory signalling, autophagy, vesicular trafficking and cell death. However, its exact neuroprotective mechanism is unclear. To assess the possible role of optineurin in neuroinflammatory signalling we made an optineurin insufficiency mouse model with loss of function truncation of ubiquitin-binding region (Optn470T). We found that in primary microglia optineurin was dispensable for NF-kappaB activation and TNF secretion upon Toll receptor stimulation (TLR) with lipopolysaccharide (LPS). However, upon stimulation with LPS and poly (I:C), Optn470T microglia had diminished Tank-binding kinase 1 (TBK1) activation, phosphorylation of interferon regulatory factor 3 (IRF3) and production of IFN-beta. Optn470T microglia also had diminished activation of transcription factor STAT1, and diminished expression of IFN-beta-regulated pro- and anti-inflammatory genes including IRF7, NOS2, CXCL10, IL-10 and CXCL1. Expression of these genes was rescued upon supplementation of recombinant IFN-beta, indicating that ubiquitin-binding function of optineurin and IFN-beta-mediated signalling are necessary for optimal microglial inflammatory response. Diminished TBK1 activation was also found in Optn470T primary neurons. Despite the in vitro differences between wild type (WT) and Optn470T models, an acute LPS challenge in vivo triggered comparable microgliosis in both genotypes. However, the brains of LPS challenged Optn470T mice had higher levels of granulocyte chemoattractants including G-CSF, CXCL1 and LIX than WT mice. Notably, unmanipulated aged Optn470T showed no signs of microgliosis in the brain or spinal cord. We speculate that additional stimuli (infectious or other) are needed for disease manifestation in the Optn470T model.

PRECISION MEDICINE:

1938: **Discussing personalized prognosis in ALS: What can we learn from other life-limiting diseases?**

Remko M. van Eenennaam* (1,2), Willeke Kruithof (1), Michael A. van Es (2), Esther Kruitwagen-van Reenen (1), Henk-Jan Westeneng (2), Anne Visser-Meily (1), Leonard H. van den Berg (2), Anita Beelen (1,2).
1) Centre of Excellence for Rehabilitation Medicine, Brain Center Rudolf Magnus, University Medical Center Utrecht, and De Hoogstraat Rehabilitation, Utrecht, The Netherlands.
2) Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands.

Background: Life expectancy of patients diagnosed with amyotrophic lateral sclerosis (ALS) varies greatly. A recently published personalized prediction model for ALS makes it possible to reliably predict survival of individual patients at diagnosis (Westeneng et al, 2018). However, discussing personalized prognosis appropriately and effectively is a challenge and a subject on which current ALS guidelines lack recommendations.
Objectives: In order to develop recommendations for discussing the personalized prognosis in ALS, we aimed to summarize evidence about 1) the effect of discussing prognosis in patients suffering from life-limiting diseases and 2) patient needs for discussing prognosis in a life-limiting disease.
Methods: A systematic review was conducted in PubMed and Google Scholar from 2000 to January 16th 2019. References of included studies and guidelines on communicating prognosis were also checked. A qualitative synthesis of results from the included studies was performed. A thematic synthesis was used to identify themes emerging from the data in qualitative studies.
Results: 13 studies were included, six of which had a qualitative design. Quantitative analyses showed no negative effect of prognostic discussion on mental distress and patient-physician relationship. Over time, discussing prognosis resulted in lower anxiety and improved patient-physician relationship. Thematic synthesis identified the theme of patient readiness, which functioned as a mediator for the effect of discussing the prognosis on the patient. Themes for patient needs were honest communication with empathy, initiated by a trusted physician, the presence of family for support, and hope-giving through emphasis on holistic nature of palliative care, reassurance of non-abandonment, and hope-giving stories.
Conclusion: Based on evidence from other life-limiting diseases, there is no reason to expect that discussion of prognosis has a negative impact on patients with ALS. However, an important prerequisite is adherence to patient readiness. We recommend tailoring the discussion of prognosis in ALS to the readiness and needs of the individual patient and discussing it in a manner that combines honesty with empathy and hope-giving.
Westeneng, H-J., Debray, T.P.A., Visser, A.E., van Eijk, R.P.A., …, van den Berg, L.H. (2018). Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurology, 17(5), 423-433

1918: **Increasing ALS Clinical Trial Efficiency using Machine Learning Models**.

Danielle Beaulieu, Albert A. Taylor, Jonavelle Cuerdo, Andrew Conklin, Dustin Pierce, Mike Keymer and David L. Ennist*
Origent Data Sciences, Inc., Washington DC, USA.

We previously developed regression models for total ALSFRS-R score, ALSFRS-R sub-scores, vital capacity (VC), and percent expected vital capacity, and time-to-event models for loss of speech, use of wheelchair, gastrostomy, use of NIV (using time to 50% expected VC as a surrogate) and survival.

ALS models were developed using the PRO-ACT database. We initially clean the data, run a preliminary random forest model for variable reduction, then refine and improve the models using gradient boosting machines. The models are validated for internal consistency using ten-fold cross validation and generalizability using external datasets. Regression models were characterized using root-mean square deviation (RMSD) and bias analysis and time-to-event models were characterized using receiver operating characteristic (ROC) curves, calibration (log-rank) and discrimination (C-index). The models were used to stratify patients and create drug development tools that were assessed using simulated trials with randomly drawn PRO-ACT data.

The models were utilized to create drug development applications, including a novel method of subgroup analysis, "detectable effect cluster analysis" (DEC analysis) to identify subgroups with significant effect sizes. In addition, we used predicted outcomes to develop improved tools for enrichment, randomization and covariate adjustment. Finally, we developed virtual controls for situations without a placebo arm (e.g., in early phase trials) and where a placebo is not possible, ethically challenging (e.g., cell & gene therapies with invasive procedures) or impossible to blind.

The detectable effect cluster (DEC) analysis, enrichment, randomization/covariate and virtual control tools find "hot spots" of patients with demonstrable benefit, decrease trial heterogeneity, lower sample size/increase power, and provide an objective measure of efficacy for drug development trials in ALS. DEC analysis shows great promise in identifying subgroups within a failed trial that could have formed a successful trial. These applications represent a significant paradigm shift with broad implications for the conduct of trials in ALS in particular and can be extended to a range of neurodegenerative diseases.

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THERAPEUTICS:

1910: **Therapeutic targeting of proteostasis in MND and FTD - a systematic review and meta-analysis of preclinical research**

Elizabeth Elliott (1,2,3*), Olivia Bailey(1,2,3), Fergal M. Waldron(4), Siddharthan Chandran(1,2,3) and Jenna M. Gregory(1,2,3)
1 Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK.
2 Euan MacDonald Centre for Motor Neurone Disease Research, University of Edinburgh, Edinburgh, UK.
3 Anne Rowling Regenerative Neurology Clinic, University of Edinburgh, Edinburgh, UK.
4 Institute of Evolutionary Biology and Centre for Immunity Infection and Evolution, University of Edinburgh, Edinburgh.

Motor Neurone Disease (MND) is a genetically and phenotypically heterogeneous condition which explains, in part, why research efforts over the last 20 years have failed to identify an effective treatment for this devastating condition. Moreover, MND is now recognised to exist on a neuropathological spectrum with frontotemporal dementia. Despite this significant clinical heterogeneity, protein misfolding has been identified as a unifying pathological feature in the majority of these cases. On the basis of this shared pathophysiology, we carried out a systematic review and meta-analysis to assess the therapeutic efficacy of compounds that target protein misfolding in preclinical studies of MND and FTD.
Systematic review identified 70 preclinical studies investigating the effects of therapies targeting protein misfolding on survival. Meta-analysis revealed that targeting protein misfolding did significantly improve survival compared to untreated controls ($p<0.001$, (df)=68, alpha = 0.05, (CI) 0.106-1.16), with no evidence of heterogeneity between studies (I squared= 0%). Further subgroup analyses, evaluating the effect of timing of these interventions, showed that, only treating prior to symptom onset (n=33), significantly improved survival ($P<0.001$, df=31, alpha = 0.05, (CI) 0.108-1.29), although this may simply reflect the inadequate sample size of later time points. Furthermore, arimoclomol was found to significantly reduce secondary outcome measures including: (i) histological outcomes, (ii) behavioural outcomes and (iii) biochemical outcomes ($p<0.005$). This analysis supports the hypothesis that protein misfolding plays an important role in the pathogenesis in MND and FTD and that targeting protein misfolding, at least in preclinical models, can significantly improve survival, especially if such an intervention is administered prior to symptom onset.

1912: **Low-dose IL-2 administration in ALS: understanding the transcriptional response to treatment**.

Ilaria Giovannelli* (1), Paul Heath (1), Pamela J Shaw (1), Janine Kirby (1).
(1): Sheffield Institute of Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom.

Background: Neuroinflammation is one of the hallmarks of amyotrophic lateral sclerosis (ALS). Recently, a key role of regulatory T cells (Tregs) in this disease was demonstrated. In ALS patients, Tregs appear to be dramatically and progressively decreased in number and less effective in promoting immune suppression during the disease progression. Moreover, Tregs levels inversely correlate with rate of ALS progression and life expectancy. IL-2 is a crucial mediator of Treg differentiation and survival. Evidence shows that low-dose-IL-2 (ld-IL-2) can selectively promote Tregs expansion. We hypothesise that ld-IL-2 treatment can mediate a specific Tregs amplification and activation leading to a decrease in neuroinflammation and increased survival.

Aim: To determine the effect of ld-IL2 on ALS patients' blood transcriptome as part of the IMODALS clinical trial.
Methods: 36 patients received 1MIU or 2MIU-IL-2 or placebo subcutaneously once daily for 5 days every 28 days for 3 months. At Day 1 (baseline), Day 64 (after 3 cycles of treatment) and Day 85 (1 month after final treatment), blood was collected and peripheral blood mononuclear cells were isolated. Subsequently, total RNA was extracted and microarrays analysis performed. Data were analysed TAC and Qlucore. qPCRs were performed to validate differentially expressed genes.
Results: Our findings demonstrate a robust Treg activation and expansion in response to treatment in a dose-dependent manner. Moreover, pathway analysis revealed the activation of key Treg mediators in treated patients. In a longitudinal analysis comparing patients' gene expression at different time points, a significant upregulation of several anti-inflammatory genes was demonstrated together with a relevant downregulation of pro-inflammatory mediators. Four key Treg genes (FOXP3, CTLA4, IL2RA and IKZF2) were validated by qPCR analysis. Even though there was variablity in terms of patient response, we observed an increase in the expression of these genes in treated patients which further demonstrate Treg activation.

1916: **Could Conservative Iron Chelation Lead to Neuroprotection in Amyotrophic Lateral Sclerosis?**

Jonneaux A(1)*, Timmerman K(1), Moreau C(2), Danel V(2), Devedjian JC (1), Grolez G(2), Laloux C(1), Petrault M(1), Gouel F(1), Dutheil M(1), Lachaud C(1), Rolland AS (1), Lopes R(3), Kuchcinski G(3), Auger F(4), Kyheng M(5), Duhamel A(5), Perez T(6), Pradat PF(7), Blasco H(8), Veyrat-Durebex C(8), Corcia P(8), Oeckl P(9), Otto M(9), Dupuis L(10), Garçon G(11), Defebvre L(2), Cabantchik I(12), Duce J(13,14), Bordet R(1), Devos D(1,2)
(1) Département de Pharmacologie Médicale, Université de Lille, INSERM UMRS_1171, Centre Hospitalier Universitaire, Centre COEN LICEND, Lille, France
(2) Département de Neurologie, Centre ALS, Université de Lille, INSERM UMRS_1171, Centre Hospitalier Universitaire, Centre COEN LICEND, Lille, France
(3) Département de Neuroradiologie, Université de Lille, INSERM UMRS_1171, Centre Hospitalier Universitaire, Centre COEN LICEND, Lille, France
(4) Département de radiologie pré-clinique, Université de Lille, INSERM UMRS_1171, Centre COEN LICEND, Lille, France
(5) Université de Lille, CHU Lille, EA 2694- Santé publique : épidémiologie et qualité des soins, Lille, France
(6) Département de pneumologie, Université de Lille, Centre Hospitalier Universitaire, Lille, France
(7) Laboratoire d'Imagerie Biomédicale, Sorbonne Universités, UPMC Univ Paris 06, CNRS, Inserm, Paris ; Département de Neurologie, AP-HP, Hôpital Pitié-Salpêtrière, Paris, France
(8) Laboratoire de biochimie, Université François Rabelais, INSERM U930, CHRU Tours, France
(9) Department of Neurology, Ulm University Hospital, Center for Biomedical Research, Ulm, Germany
(10) INSERM UMR-S1118, Faculté de Médecine de Strasbourg, France
(11) Université de Lille, CHU Lille, Institut Pasteur de Lille, EA 4483 IMPECS-IMPact de l'Environnement Chimique sur la Santé humaine, France
(12) Della Pergola Chair, Alexander Silberman Institute of Life Sciences, Hebrew University, Jerusalem, 91904, Israel
(13) Alzheimer's Research UK Cambridge Drug Discovery Institute, University of Cambridge, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0AH, UK.
(14) The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia.

Iron accumulation has been observed in mouse models and in both sporadic and familial forms of amyotrophic lateral sclerosis (ALS). Iron chelation could reduce iron accumulation and the related excess of oxidative stress in the motor pathways. However, classical iron chelation would induce systemic iron depletion. We assess the safety and efficacy of

conservative iron chelation (i.e., chelation with low risk of iron depletion) in a murine preclinical model and pilot clinical trial. In Sod1G86R mice, deferiprone increased the mean life span compared with placebo. The safety was good, without anemia after 12 months of deferiprone in the 23 ALS patients enrolled in the clinical trial. The decreases in the ALS Functional Rating Scale and the body mass index were significantly smaller for the first 3 months of deferiprone treatment (30 mg/kg/day) than for the first treatment- free period. Iron levels in the cervical spinal cord, medulla oblongata, and motor cortex (according to magnetic resonance imaging), as well as cerebrospinal fluid levels of oxidative stress and neurofilament light chains were lower after deferiprone treatment. Our observation leads to the hypothesis that moderate iron chelation regimen that avoids changes in systemic iron levels may constitute a novel therapeutic modality of neuroprotection for ALS.

1921: **A new therapeutic approach in Amyotrophic Lateral Sclerosis based on the human platelet lysate.**

Gouel F (1)*, Timmerman K(1), Dutheil M(1), Jonneaux A(1), Laloux C (1,3), Moreau C (2), Rolland AS (1), Danel V (2), Bordet R(1), Devedjian JC(1), Devos D(1,2).
(1) Département de Pharmacologie médicale, INSERM UMRS 1171, Université de Lille, CHU de Lille
(2) Département de Neurologie, Université de Lille, CHU de Lille
(3) Plateforme d'exploration fonctionnelle, SFR DN2M

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that typically results in death within 3 to 5 years after diagnosis. To date, there is no curative treatment and therefore and urgent unmet need of neuroprotective and/or neurorestorative treatments. Due to their spectrum of capacities, neurotrophic growth factors (NTF) have been exploited for therapeutic strategies in ALS for decades. Despite the promising effects of NTF treatment in preclinical animal models of ALS, only disappointing results were obtain in clinical trials. However, we hypothesize than administration of a mix of pleiotrophic neurotrophic factors may be crucial to power the neuroprotection/neurorestoration therapy requested in this devastating disease. To this end, we focus on a new biotherapy based upon intracerebral administration of human platelet lysate, natural healing system source of numerous NTFs, which has demonstrated neuroprotective effect in ischemic rats. We therefore developed and patent three processes for the preparation and activation of human purified platelet lysates (PPLs), compatible with intracerebroventricular administration, subjected to heat treatment (HPPL) to deplete fibrinogen, avoid thrombogenic and proteolytic activities. We first tested HPPL in vitro, on dopaminergic neurons intoxicated with the parkinsonian drug MPP+ and on a motoneuron-like model. A high neuroprotection as well as protection against apoptosis and oxidative stress was observed. Based on these results, HPPL was then tested with intranasal or intracerebroventricular administration in SOD1G68R mice, an ALS animal model. Both administration routes were well tolerated and a delay onset as well as an increased in the lifespan was observed. To then seek to determine the neuroprotective elements within the lysate, we fractionated the HPPLs according to the size and tested it in vitro and in vivo. Surprisingly, a 3 KDa fraction protects as much as the HPPLs in human dopaminergic cells and shows a delay onset and increased survival of SOD1G68R mice. All together, these results are supportive .

1926 **ROCK-ALS: a phase IIa clinical trial evaluating inhibition of Rho kinase (ROCK) with Fasudil as disease-modifying treatment for ALS.**

Paul Lingor* (1,2), Markus Weber (3), William Camu (4), Magdalena Kuzma-Kozakiewicz (5), Tim Friede (6), Jan C. Koch (2).
1 Rechts der Isar Hospital of the Technical University of Munich, Munich, Germany.
2 University Medical Center Göttingen, Department of Neurology, Göttingen, Germany.

3 Kantonsspital St. Gallen, Muskelzentrum/ALS Clinic, St. Gallen, Switzerland.
4 Centre de Référence Maladie Rares SLA et autres maladies rares du neurone moteur, INSERM 1051, CHU Gui de Chauliac, Montpellier, France.
5 Department of Neurology, Medical University of Warsaw, Warsaw, Poland.
6 University Medical Center Göttingen, Department of Medical Statistics, Göttingen, Germany.

Background: An effective disease-modifying therapy for ALS is still not available. Two drugs are currently in clinical use, Riluzole and Edaravone, but their clinical effects are moderate. The Rho kinase (ROCK) inhibitor Fasudil has demonstrated neuroprotective effects on motor neuron survival in preclinical studies, has been shown to enhance axonal regeneration and to modulate microglial function in vitro and in vivo. Fasudil has been approved in Japan for many years for the treatment of vasospasms following subarachnoid hemorrhage and has a favorable side effect profile.
Objectives: To develop a safe and tolerable new disease-modifying therapy for ALS patients based on repurposing of Fasudil.
Methods: ROCK-ALS is a phase IIa clinical trial investigating the safety, tolerability, and efficacy of Fasudil in ALS patients at an early stage of disease (EudraCT-Nr.: 2017-003676-31, NCT03792490). Safety and tolerability will be primary endpoints. Efficacy is a secondary endpoint and will be assessed by the change in ALSFRS-R, ALSAQ-5, vital capacity, MUNIX as well as overall survival. In addition, the study will collect biomarker fluids (blood, cerebrospinal fluid, saliva and urine) for the correlation of surrogate parameters with clinical outcomes. The trial is expected to enroll a total of 120 patients with a probable or definitive ALS (according to the revised El Escorial criteria) and a disease duration of at least 6 but not more than 24 months. A total of 16 centers in Germany, Switzerland and France are participating in this interventional, randomized study as part of an international consortium. An associated center in Poland will be recruiting patients in an independent, untreated biomarker cohort. In a three-arm design with 1:1:1 treatment allocation, patients will receive an intravenous dose of either 15 mg fasudil, 30 mg fasudil or placebo twice daily for a total of 20 days. Safety-relevant examinations will be carried out during the entire treatment period as well as in the follow-up period until day 180. Efficacy-relevant examinations will be performed before and immediately after the infusion therapy as well as on days 42, 84 and 180.
Results: The ROCK-ALS trial is funded under the 2016 EU E-Rare program (http://erare.eu/financed-projects/rock-als). All regulatory approvals were obtained and first patient first visit was achieved in early 2019.

1930: **Characterization of macrophage migration inhibitory factor as a therapeutic target for amyotrophic lateral sclerosis.**

Leenor Alfahel*, Marcel Leyton, Joy Kahn, Adrian Israelson.
Department of Physiology and Cell Biology, Faculty of Health Sciences and the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Israel.

Mutations in superoxide dismutase 1 (SOD1) cause amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the loss of upper and lower motor neurons. Transgenic mice expressing mutant SOD1 develop paralysis and accumulate misfolded SOD1 onto the cytoplasmic faces of intracellular organelles, including mitochondria and endoplasmic reticulum (ER). We have shown that macrophage migration inhibitory factor (MIF) is able to directly inhibit mutant SOD1 misfolding and binding to intracellular membranes. In accordance, complete elimination of endogenous MIF accelerates disease onset and late disease progression, as well as shortens the lifespan of mutant SOD1 mice with higher amounts of misfolded SOD1 detected within the spinal cord.

Based on these findings, we used adeno associated viral (AAV) vectors to overexpress MIF in the spinal cord of mutant SOD1G93A and loxSOD1G37R mice. Our data show that MIF mRNA and protein levels were increased in the spinal cords and brains of AAV2/9-MIF injected mice. Furthermore, mutant SOD1G93A and loxSOD1G37R mice injected with AAV2/9-MIF demonstrated a significant delay in disease onset and prolonged survival compared with their AAV2/9-GFP injected or non-injected littermates. Moreover, these mice accumulated reduced amounts of misfolded SOD1 in their spinal cords, with no observed effect on glial overactivation as a result of MIF upregulation.
Of more therapeutic relevance, would be to inject MIF at the symptomatic stage of the disease, when disease diagnosis has been established. To this end, we utilize an AAV PHP.eB virus that has the ability to penetrate the blood-brain barrier (BBB), following peripheral injection at the symptomatic phase. We expect that injecting MIF at this stage, will delay disease progression and expand the lifespan of mutant SOD1 mice.
Our findings indicate that MIF acts as a chaperone for misfolded SOD1 in vivo and may have further implications regarding the therapeutic potential role of upregulation of MIF in modulating the specific accumulation of misfolded SOD1.

1940: **Compassionate use of the ROCK inhibitor Fasudil in three patients with amyotrophic lateral sclerosis**

Jan C. Koch, Eirini Zielke, Jeanneke Spruit, Josua Kuttler, Paul Lingor.
University Medical Center Göttingen, Department of Neurology, Göttingen, Germany

The Rho kinase (ROCK) inhibitor Fasudil is a promising drug for a disease-modifying therapy of amyotrophic lateral sclerosis (ALS). In preclinical models, Fasudil was shown to increase motor neuron survival, inhibit axonal degeneration, enhance axonal regeneration and to modulate microglial function in vitro and in vivo. It prolonged survival and improved motor function of SOD1-G93A-mice significantly. A phase IIa clinical trial has now been commenced to investigate the safety, tolerability, and efficacy of Fasudil in ALS patients at an early stage of disease (ROCK-ALS trial, EudraCT-Nr. 2017-003676-31). Although Fasudil has been approved in Japan for many years for the treatment of vasospasms following subarachnoid hemorrhage and is known to have a favorable side effect profile in these patients, it has so far never been given to human patients with ALS or any other neurodegenerative condition.
Here, we report the first three cases of compassionate use of Fasudil in patients with ALS. Between May 2017 and February 2019, one male (66 years old) and two female (62 and 68 years old) patients with probable ALS according to the El Escorial criteria were treated with Fasudil 30 mg intravenously twice daily on 20 consecutive working days. Riluzole was taken as co-medication by all three patients. Blood pressure, heart rate and routine laboratory tests were monitored. Clinical examination, ALSFRS-R, vital capacity and MUNIX were assessed at multiple time-points. All three subjects tolerated the Fasudil infusions well without any obvious side effects. Interestingly, the slow vital capacity in one of the patients showed a significant increase over the infusion period that persisted over one month thereafter.
Taken together, we report here the first compassionate use of the ROCK inhibitor Fasudil in three ALS patients, which was well tolerated.

1953: **The Development of Intrabodies for Therapeutics Targeting TDP-43 Aggregation in ALS.**

Rudolf Hergesheimer*(1), Anna Chami (1), Patrick Vourc'h (1,2), Christian Andres (1,2), Philippe Corcia (1,3), Pierre Martineau (4), Denis Reis de Assis (1), Débora Lanznaster (1), Hélène Blasco (1,2).

1. UMR 1253, iBRAIN, Université de Tours, INSERM, Tours, France.
2. CHU de Tours, Service de Biochimie et Biologie Moléculaire, Tours, France.
3. CHU de Tours, Service de Neurologie, Tours, France.
4. UMR 896, IRCM, INSERM, Montpellier, France

Few drugs have a significant clinical impact on patients suffering from ALS, thus leading to active research in drug development and biomarker exploration to follow therapeutic trials. One of the most attractive putative targets is the DNA/RNA-binding protein TDP-43. While mostly nuclear in physiological conditions, the wild-type form is found in cytoplasmic aggregates in 95% of ALS patients. TDP-43 aggregation is now considered the hallmark of ALS, and this justifies efforts to prevent aggregation as a neuroprotective strategy. The aim of our study was to find innovative molecules preventing TDP-43 aggregation via the use of biopharmaceuticals. Here, we purified under denaturing conditions soluble, recombinant GFP-wtTDP-43-Histag overexpressed in HEK293T cells. A library of small-chain variable fragments (scFv) was screened by phage-display to select for potentially TDP-43-specific scFvs . After selection, sandwich ELISA assays revealed several clones (> 8) that exhibited specificity towards TDP-43, and at least 4 of these clones displayed distinct sequences, revealing a convergence during selection. Four unique scFvs were then cloned into the pET23NN vector for their purification from BL21(DE3) E. coli and also cloned into the pDsRed-Monomer-C In-Fusion vector for their expression in mammalian cells. Using these plasmids, we are currently validating the following parameters of each scFv in order to determine their functionality: scFv solubility in HEK293T cells, co-localization with aggregated and soluble forms of TDP-43 in cells, and inhibition of TDP-43 aggregation in vitro and in cells. Once these criteria are met, we will determine the therapeutic ability of the candidate scFvs by measuring the changes in multiple cellular parameters that are affected by TDP-43 overexpression, such as mitochondrial metabolism, calcium balance, oxidative stress, neurite length, and cellular viability in HEK293T cells, Neuro2A cells, and ALS patient fibroblasts.
Combining currently available therapy with inhibitors of TDP-43 aggregation could yield very beneficial outcomes for ALS patients. Most importantly, the emerging strategy of using intrabodies to develop new therapeutics has shown considerable efficiency and could be used for further developments in other neurological disorders."

1957: **Antioxidant drugs reveal the potential for patient stratification in Motor Neurone Disease**

Chloe Allen (*1), Monika Myszczynska (1), Matthew Stopford (1), Noemi Gatto (1), Heather Mortiboys (1), Guillaume Hautbergue (1), Pamela J Shaw (1) and Laura Ferraiuolo (1).
(1) Sheffield Institute for Translational Neuroscience, University of Sheffield (SITraN), Sheffield, UK.

Current medicine is moving away from a 'one size fits all' approach and instead focusing on how individual genetic characteristics determine treatment response. Therapeutic approaches towards ALS have always proved difficult due to the impressive genetic and phenotypic heterogeneity of the disease, indicating a need for a precision medicine approach. A previous study at SITraN testing the effect of antioxidant drugs against patient-derived astrocyte toxicity found that specific drugs were more effective in astrocytes harbouring specific mutations.
The aim of this study is to decipher genetic signatures that will discriminate between patient responders to specific antioxidant drugs as well as investigating drug response at a cellular level.

Astrocytes were generated from three unaffected individuals and three different patient subgroups of ALS (n=3 per group) to identify the modes of action of Riluzole as well as two antioxidant drugs, Andrographolide and Compound A*.
A combination of immunofluorescence approaches alongside RNA-sequencing of the poly-adenylated RNA reported significant differences in the accumulation of misfolded SOD1 aggregates, p62 expression, TDP-43 mislocalisation and mitochondrial dysregulation in patient astrocytes, reflecting reports from patient post-mortem tissue. KEGG pathway analysis of the RNA-seq data demonstrated differently regulated pathways in patient astrocytes over controls and these pathways were specific to the subgroup; large upregulation in pro-inflammatory pathways in SOD1 astrocytes while C9ORF72 pathways focused on RNA metabolism and transport.
Translatome profiling of the patient astrocytes after drug treatment has highlighted specific differences in the drug response, some of which have been confirmed with the previous immunofluorescence techniques. Further RNA-seq analysis will be used to determine genetic signatures that will discriminate between patient responders and non-responders to the antioxidant drugs, driving personalised medicine in ALS.
*Compound A currently undergoing patent

1958: **Long term follow-up of intrathecal baclofen therapy of spasticity in motor neuron disease (MND)**

Kathi Schweikert (1, 2*), Stephanie Wilmes (1), Christian Kätterer (1), Morten-Goetz Wasner (3), Gregory Jost (4), Christoph Neuwirth (5), Markus Weber (5)
1 REHAB Basel, Switzerland,
2 Neuromuscular Center, University Hospital Basel, Switzerland,
3 Spine Center, University Hospital Basel, Switzerland,
4 Spinal Surgery, University Hospital Basel, Switzerland,
5 Muscular Disease Unit/ALS Clinic Kantonsspital St. Gallen, Switzerland.

Background: Treatment of severe spasticity in MND is often unsatisfactory due to intolerance or inefficacy of oral medication. In primary lateral sclerosis (PLS) and upper-motor neuron predominant amyotrophic lateral sclerosis (ALS), intrathecal baclofen (ITB) therapy can be a therapeutic option but long term effect is unclear.
Objectives: To report on long term follow-up of MND patients under ITB for severe spasticity in Switzerland
Methods: A total of 22 MND patients were referred by ALS clinics for evaluation of ITB therapy. The effect of ITB was tested by a probatory external pump, connected with a subcutaneous intrathecal catheter, placed about 40-60 cm above L3/L4 puncture level. ITB dosage was increased according to clinical signs, oral antispastic medication tapered off and stopped. Spasticity was evaluated daily by modified Ashworth scale. Patients who went on ITB therapy were followed in ALS clinics every 3 months. ALSFRS-R, ITB dosage, additional spasticity treatment was documented.
Results: From 2/2007 to 3/2019, eighteen ALS and three PLS patients (15 men, 6 women), mean age 54.6 years, were evaluated for ITB via probatory external pump. Mean disease duration preoperatively was 58.6 months, mean ALSFRS-R at baseline 28. In all patients spasticity was reduced by ITB, no side effects were reported. Five patients did not go on permanent ITB therapy as motor function deteriorated or symptoms did not improve. A pump (Synchromed II, Medtronic) was implanted in 16 patients. Mean ITB dose after first titration was 52.6 ug/d and ITB application continued in all patients. One patient was lost to follow-up after two months. Ten patients died, four of them by assisted suicide, six by respiratory failure due to ALS. In those patients mean duration of ITB therapy was 31.5 months (range 2 to 114), and 44.4 months (24 to 73) in the patients still alive. At the last

evaluation mean ALSFRS-R was 16.3, and 26.8, mean ITB dose 110.4 ug/d (40 to 413), and 124 ug/d (44 to 193) respectively. Nine patients needed additional antispastic medication.
Discussion: In MND patients the pattern of muscle tone and strength varies substantially. Severe spasticity might require ITB therapy, but progression of atrophic paresis and spasticity must be considered.
Conclusions: ITB can safely and effectively reduce spasticity in long term course of selected MND patients. Escalation of ITB dose was mandatory in most of our patients, additional antispastic medication in 60% of cases.

1973: **Nuclear factor erythroid 2-related factor 2 as a gene modulator of response to oxidative stress in amyotrophic lateral sclerosis** .

Annalisa Lo Gerfo *(1), Costanza Simoncini (1), Lucia Chico (1), Vincenzo Montano (1), Cecilia Carlesi (1), Filippo Baldacci (1), Simona Daniele (2), Maria Rosaria Maluccio (3), Giulia Ricci (1), Michelangelo Mancuso (1) and Gabriele Siciliano (1)
1 Department of Clinical and Experimental Medicine, University of Pisa, 56100 Pisa, Italy.
2 Department of Pharmacy, University of Pisa, 56100 Pisa, Italy.
3 Clinic of Neurology, Department of Clinical Medicine, ATNO, Italy.

Among the pathogenic mechanisms on motor neuron degeneration leading to amyotrophic lateral sclerosis (ALS), the oxidative stress (OS) theory has been put forward. Although it remains hard to understand whether or not OS is the cause or effect of the disease, the association between oxidative damage and the disease makes therapeutic targeting of the antioxidant systems an attractive option. NRF2-ARE pathway is a primary sensor and a master regulator of OS via its ability to modulate the expression of hundreds of antioxidant genes.
Considering the pivotal defensive role exerted by the Nrf2/ARE pathway (demostrated in animal models of many neurodegenerative disorders), it is evident that the dysregulation of Nrf2-regulated genes offers a possible explanation for the direct and indirect association between OS and ALS.
This work is aimed to evaluate a possible association between -653 A> G, -651 G> A and -617 C> functional polymorphisms in the NRF2 promoter gene with the NRF2 mRNA and oxidative stress biomarkers in ALS.
Analysis of 150 ALS patients data shows that the allelic -653G variant is associated with increased risk of disease (OR 1.71 IC95% 1.18-2.48); in relation to the polymorphisms -651 G> A and -617 C> A, no significant differences have been found either in the genotypic distribution or in the allelic frequencies of patients with ALS compared to the controls. The evaluation of peripheral oxidative stress biomarkers shows a significant increase in AOPP levels ($p< 0.001$) and a significant decrease in thiol groups levels (p0.05) compared to wild-type (AA) carriers at this position. Finally, the data obtained show a correlation between -653G variant, mRNA expression level and oxidative stress biomarkers in ALS patients.
The data obtained suggest that the -653G variant in Nrf2 promoter gene can be a risk factor for ALS. This variant is associated to decreased level of Nrf2 mRNA as evaluated in peripheral lymphocytes.
All together these data reinforce the statement that Nrf2-ARE pathway can be one of the pathogenic molecular mechanisms to be considered in motoneuron neurodegeneration in ALS. Conclusive remarks can be assumed in terms of relevance of oxidative stress events as integral part of the pathogenic complex of this disease

1976: **Adipose Derived Stem Cells for cell theraphy of Motor Neuron Disease.**

Yuri Ciervo (*) (1,2), Ian Coldicott (1), Monika Myszczynska (1), Matthew Stopford (1), Chloe Allen (1), Andrew Grierson (1), Jun Xu (2), Laura Ferraiuolo (1), Ke Ning (1,2), Pamela Shaw (1), Richard Mead (1)

(1) Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom.
(2) Department of Regenerative Medicine, Tongji University, Shanghai, China.

Cell-based therapy is a promising therapeutic strategy in ALS. Adipose Derived Stem Cells (ADSC) have the potential to target multiple disease pathways through immunomodulation, growth factor secretion and neuroprotection. These cells have the potential to provide therapeutic benefit when delivered directly into cerebrospinal fluid via modulation of the toxic environment leading to motor neuron degeneration. We first investigated the therapeutic potential of intrathecal transplantation of mouse ADSC in pre-symptomatic SOD1G93A mice. Mice were injected with GFP-ADSC or with PBS alone into the cisterna magna and motor function evaluated using rotarod performance and gait analysis, weight measured and onset of disease assessed by neuroscoring. Tissue was collected at several time points to determine engraftment and glial activation. ADSC injection into SOD1G93A mice resulted in significant weight gain, delay of motor function decline by 2 weeks and later clinical onset (5 days) compared to PBS injected mice. Histological examination showed that ADSCs can survive in the ventricular system and spinal cord meninges of SOD1G93A mice and are able to attenuate glial activation. We then tested the neuroprotective potential of mouse ADSCs in astrocyte/motor neuron (MN) co-cultures (patient and mouse model derived astrocytes) where ALS astrocytes show toxicity. We performed separated co-culture experiments between HB9-GFP-MN, ADSC and WT or SOD1G93A cortical astrocytes or with human induced astrocytes derived from ALS patients and healthy controls. ADSCs were able to rescue MN death caused by ALS astrocyte toxicity, both from SOD1G93A mouse astrocytes and human iAstrocytes derived from ALS patients with both familial and sporadic disease. ADSCs significantly reduced the secretion of inflammatory cytokines (Il-6, MCP-1, IL-1_ and TNF-_) from SOD1G93A astrocytes and increased the expression levels of neurotrophic factors. In conclusion we show that intrathecal transplantation of ADSC is beneficial to SOD1G93A mice. In vitro, ADSCs were able to protect MNs from ALS-linked astrocyte toxicity through paracrine effects in part explaining the beneficial effect observed in vivo where ADSC did not migrate into the CNS parenchyma. For the first time, we demonstrate potential therapeutic translation of ADSC into human ALS by using astrocytes derived from patients. Overall these data in two translational models of ALS point to the therapeutic potential of ADSCs for this disease.

1988: **Safety and tolerability of oral levosimendan.**

Matti Kivikko (1), Toni Sarapohja (1), Valtteri Aho (2)(*)
(1) Orion Pharma, Orion Corporation, Espoo, Finland.
(2) Orion Pharma, Orion Corporation, Turku, Finland.

Background: Intravenous levosimendan is in clinical use for acute heart failure in nearly 60 countries worldwide. As a calcium-sensitizer it has shown to improve force generation of both slow and fast diaphragm muscle fibres and cardiac muscle fibres. Recently, positive effects of oral levosimendan on supine slow vital capacity (SVC) in patients with amyotrophic lateral sclerosis (ALS) were seen in the LEVALS pilot study. Thus far, no detailed safety data for long-term oral levosimendan treatment are available.
Methods: The relevant safety data from previous studies with oral levosimendan and their relation to intravenous (IV) levosimendan was analysed. Here, the data are presented from studies conducted in patients with chronic heart failure (median exposure 6 months) (n=307), patients with recent stroke or transient ischemic attack (TIA)(n=21), and from the LEVALS study in patients with ALS (up to 7 months)(n=66).
Results: The most frequent adverse event (AE) with oral levosimendan in patients with ALS was headache, occurring in 17%, 29% and 3% of patients with levosimendan 1 mg daily, 2

mg daily and placebo, respectively. Increased heart rate was reported more frequently in patients receiving levosimendan 1 mg or 2 mg daily than placebo; 5%, 19% and 2%, respectively. In patients with chronic heart failure increased heart rate was more frequent with levosimendan 1 mg and 2 mg daily than with placebo; 11%, 15% and 3%, respectively. In addition, headache was more commonly seen with levosimendan than with placebo; 4%, 7% and 1%, respectively.
With IV levosimendan (mainly in acute heart failure), the clinically most important AEs are hypotension and atrial fibrillation. IV levosimendan also increases heart rate and decreases blood pressure. Increase in heart rate with constant oral levosimendan dosing was observed (up to 11 bpm with 2 mg daily dose), but the effect on blood pressure was clinically insignificant. In a few studies atrial or ventricular arrhythmias were observed with IV levosimendan but not with oral levosimendan. In ambulatory ECG recordings in patients with recent stroke or TIA and in ALS patients, no relevant arrhythmic events were noted. QTc interval was not prolonged either with IV or oral preparation.
Conclusion. Oral levosimendan is well-tolerated in long-term use in ALS and chronic heart failure. The most common adverse pharmacodynamic effects are headache and increased heart rate.

2013: **Design of the Phase 3, Randomised, Placebo-Controlled Trial of oral Arimoclomol in Amyotrophic Lateral Sclerosis ORARIALS-01** .

Claus Sundgreen (1), Thomas Blaettler (1), Richard Bennett (1) Dror Rom (2), Peter M. Andersen (3*), Michael Benatar (4).
1) Orphazyme A/S, Copenhagen, Denmark.
2) Prosoft Clinical Inc, Huntingdon Valley, PA, USA.
3) Department of Pharmacology and Clinical Neuroscience, Umeå University, Sweden.
4) Department of Neurology, University of Miami, FL, USA.

Background: Arimoclomol is an amplifier of the heat shock response under conditions of cellular stress. Heat shock response promotes clearance of intracellular protein aggregates, natural folding of nascent proteins, and refolding of misfolded proteins, reconstituting normal protein function - actions that are expected to have a disease modifying effect. A 12-month phase 2 trial of 36 SOD1 patients with aggressive disease, oral arimoclomol treatment reduced the rate of decline of the ALSFRS-R by 0.5 points/month and by 1 point/month in the subgroup of 24 patients with A4V SOD1 mutation as compared to placebo. Exploratory efficacy assessment of the open label extension part of another phase 2 trial showed a slower reduction in ALSFRS-R compared to a historical placebo control group (app. 30% difference in decline, p=0.034).
Methods: Inclusion criteria are based on analysis of the PRO-ACT database identifying patients with relatively homogenous progression over an observation period of 12–18 months. In considering survival and change in ALSFRS-R, it became evident that an observation period of 18 months would allow for more robust signal detection than 12 months. The primary endpoint is the measurement of the Combined Assessment of Function and Survival (CAFS) in the arimoclomol treatment arm as compared to placebo after 18 months. Assuming an effect size of 0.48 on CAFS, 213 patients randomised 2:1 to arimoclomol and placebo will provide 90% power to detect a statistically significant difference, at a two-sided type-1 error of 0.0446 adjusting for a group-sequential interim analysis. Secondary endpoints include PAV/tracheostomy-free survival and change in ALSFRS-R.
Results: Eligible patients are patients aged ≥18 years who meet the revised El Escorial criteria for clinically possible, clinically probable, clinically probable laboratory-supported or clinically definite ALS, or have familial ALS caused by a known pathogenic mutation. The patients will be early in their disease course, ≤18 months since first appearance of weakness,

have a baseline ALSFRS-R of ≥35 and a relatively preserved lung function with SVC ≥70% of predicted normal. Participants will be evaluated in clinic every 8 weeks for endpoints, safety measures, quality of life and biomarkers for the first 52 weeks and then every 12 weeks. To reduce the drop-out rate, patients may be assessed in their home if disease progression prevents their ability to attend the trial site.

2020: **Metabotropic glutamate receptor 5 as a potential pharmacological target in ALS.**

Tiziana Bonifacino*(1), Marco Milanese(1), Aldamaria Puliti(2,3), Claudia Rebosio(1), Francesca Provenzano(1), Carola Torazza(1), Cesare Usai(4), Giambattista Bonanno(1,5)
(1) Department of Pharmacy, Unit of Pharmacology and Toxicology, and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy.
(2) Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, Genoa, Italy.
(3) Medical Genetics Unit, Istituto Giannina Gaslini, Genoa, Italy
(4) Institute of Biophysics, National Research Council (CNR), Genoa, Italy
(5) IRCCS San Martino Policlinic Hospital, Genoa, Italy.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by muscle wasting, weakness and motor neuron (MN) death. To date, the aetiology of the disease is not clear, although glutamate (Glu)-mediated excitotoxicity represents one major cause. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in Glu-mediated excitotoxicity, since they are largely over-expressed during disease progression in ALS and involved in prominent cellular processes. In this scenario, we recently demonstrated that mGluR1 and mGluR5 at Glu nerve terminals produce abnormal Glu release (PMID22634363) and that halving mGluR1 or mGluR5 expression in SOD1G93A mice significantly prolongs survival and ameliorates disease progression as well as several biochemical, cellular and functional parameters. In parallel, we already demonstrated that the down-regulation of mGluR1 in SOD1G93A mice positively affects motor skills in both males and females (PMID24361555), while the down-regulation of mGluR5 ameliorates motor performances in males only (PMID28645622).
The aim of this work was to strengthen the impact of mGluR5 in ALS. Thus, we investigated the effects of knocking out mGluR5 in SOD1G93A mice, producing homozygous SOD1G93AmGluR5-/- mice, and of the in-vivo pharmacological treatment of SOD1G93A mice with the mGluR5 negative allosteric modulator 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP) (PMID21849627).
SOD1G93AmGluR5-/- mice showed prolonged survival probability ($p<0.001$) and delayed disease onset ($p<0.05$), accompanied by spinal MN preservation ($p<0.01$) and decreased astrocytic and microglial activation ($p<0.001$). Moreover, SOD1G93AmGluR5-/- mice showed a strong amelioration of motor skills in both males and females ($p<0.05$). Due to these encouraging results, we treated SOD1G93A mice with CTEP from 90 days of life until death, by using two different doses of the drug (2mg/kg every 48hs and 4mg/kg every 24hs). The lower dose CTEP-treated SOD1G93A mice showed a significant prolonged survival probability and improved clinical scores only in female mice ($p<0.05$). Conversely, the higher dose of CTEP produced a marked survival and clinical amelioration, both in female and male SOD1G93A mice ($p<0.05$).
These results represent a proof of concept supporting the assumption that dampening mGluR5 activity has a positive impact in ALS and make it a useful target for the pharmacological treatment of the disease.

2037: **Targeting TGF-β upregulation with an LNA-Antisense Oligonucleotide (NVP-13) to modulate the neurogenic niche for ALS therapy.**

Sebastian Peters (1), Eva Zitzelsperger (1), Sabrina Kuespert (1), Rosmarie Heydn (1), Ludwig Aigner (2), Tim-Henrik Bruun (1), Ulrich Bogdahn* (1)
1: Department of Neurology, University Hospital Regensburg, Regensburg, Germany.
2: Inst. Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria

Background: The ability of the adult central nervous system to self-repair/regenerate was described repeatedly throughout the last decades but remains in debate. In neurodegenerative disorders reduced neurogenic niche activity, in combination with neuronal loss, represents hallmarks of disease progression. As previously shown, the TGF-β system is a potential pathogenic player in Amyotrophic Lateral Sclerosis (ALS), particularly by inducing an imbalance of neurodegeneration and neuro-regeneration. The drug candidate "NVP-13" effectively reduces TGFβ-RII expression and signal transduction in vitro, paralleled by enhancing neurogenesis in human neuronal progenitor cells. In preparing a clinical translation to ALS we completed a "NVP-13" in vivo 13-week GLP Tox study with repeated intrathecal injections in Cynomolgus Monkeys.
Methods: We first tested NVP-13 tolerability in a non-GLP dose-escalation paradigm (0.4 mg/animal to 20 mg/animal). Following an NVP-13 pharmacokinetics and a pre-GLP-13-week-Tox study, we completed a GLP-13-week + 13 week recovery study in cynomolgus.
Results: In the GLP-study IT-L bolus administration has been successfully utilized to achieve CNS drug delivery with NOAEL at all dose levels up to 4 mg / animal. Plasma and CSF concentrations of NVP-13 were measured after administration of escalating doses of NVP-13. In the pivotal 13-week GLP-study reduced mRNA expression levels of the target (TGFRII) were found in spinal cord, hippocampus and SVZ. Interestingly, a positive correlation of NVP-13 concentrations in the CNS and expression of neuronal stem cell markers was found. Safety pharmacology parameters were monitored during the study and revealed no adverse effects on vital functions. Cmax and AUC0-t values in CSF and plasma did not suggest accumulation of NVP-13 after repeated IT-L administration. In specific CNS tissue samples, the neurogenic niche was effectively modulated.
Conclusion: NVP-13 is well tolerated, safe, stable and highly active to downregulate a potentially overactive TGF-β system. NVP-13 is a new pharmacological modulator of the neurogenic niche and may constitute an attractive drug candidate for ALS and further CNS disorders.

2041: **Summary of the US Safety Data for Radicava® (edaravone): Findings From Postmarketing Pharmacovigilance.**

Laura Bower, MD (1); Ming Ji, MD, MS (1); Stephen Apple, MD (2); Minh Hoang, PharmD (1); Lesly Tran, BSN, RN (1); Bernard Hahn, PharmD (1); Alexander Kalin, MA (1); Alex Kim (1)
1. Mitsubishi Tanabe Pharma Development America, Inc., Jersey City, NJ.
2. Mitsubishi Tanabe Pharma America, Inc., Jersey City, New Jersey.

Background: Postmarketing safety data have been collected since the FDA approval of edaravone (Radicava®) for amyotrophic lateral sclerosis (ALS) in May 2017. Estimated cumulative patient exposure was 3007 patients as of August 6, 2018.
Objective: Summarize the overall safety profile from real-world patient use in the US.
Methods: Cumulative patient cases and adverse events (AEs) were identified from a postmarketing safety database between Aug 8, 2017 and Aug 3, 2018 (cutoff date).
Results: From postmarketing sources in the US, there were 817 cases reported with a total of 1660 AEs, of which 391 were serious. The most commonly reported events (>50 reports) were drug ineffective, death, therapeutic response unexpected, asthenia, fatigue, gait

disturbance, disease progression, muscular weakness, fall, and dyspnea. The most commonly reported serious events (>10 reports) were death, dyspnea, pneumonia, fall, ALS, incorrect dose administration, respiratory failure, and disease progression. There were 150 cumulative death cases, with 104 cases reported as an event (not specified); however, many reports had missing information that prevented an establishment of a causal relationship with treatment. Review for serious anaphylactic reaction/hypersensitivity revealed 2 reports (1 patient). Review for serious thromboembolic events revealed 6 pulmonary embolism, 6 deep vein thrombosis, and 3 thrombosis; site reaction review revealed 9 serious reports of injection-site infection. There were 2 serious hepatic cases and 2 serious renal cases reported.
Conclusions: In the postmarketing reporting to date, no unexpected safety signals or inconsistencies with the clinical trials emerged. The limitations stemming from voluntary reporting and occasional missing information should be considered when interpreting these results.
Acknowledgments: Editorial support from p-value communications. The authors are employees of either MTDA (LB, MJ, MH, LT, BH, AK, and AK) or MTPA (SA).

2043: **Surveillance of Using Novel Free Radical Scavenger, Edaravone to Investigate Survival Effect for ALS Patients (SUNRISE Japan): Interim Report.**
Kaoru Ishizaki (1); Satoshi Yuki (2); Manabu Hirai (3); Hiroaki Matsuda (4); Gen Sobue (5)
1. Pharmacovigilance Department, Mitsubishi Tanabe Pharma Corporation, Inc. (MTPC), Tokyo, Japan.
2. Medical Intelligence Department, MTPC.
3. Clinical Research & Development I Department, MTPC.
4. Data Science Department, MTPC.
5. Brain and Mind Research Center, Nagoya University Graduate School of Medicine, Japan.

Background: Edaravone, a free radical scavenger developed as a neuroprotectant, was first approved in 2001 in Japan, indicated for acute ischemic stroke (AIS). Based on a series of clinical studies completed in Japan in patients with amyotrophic lateral sclerosis (ALS), edaravone was approved in Japan in June 2015, in South Korea in December 2015, in the United States in May 2017, in Canada in October 2018, and in Switzerland in January 2019 for the treatment of ALS. The approvals were based on the efficacy and safety data of patients with definite or probable ALS diagnosis. Edaravone demonstrated statistically significant efficacy in slowing the progression of ALS, as assessed based on the ALS Functional Rating Scale-Revised (ALSFRS-R) score; however, to date, other survival endpoints such survival time or time to tracheal intubation have not been assessed.
Objective: To assess the long-term efficacy, including survival endpoints, and safety of edaravone in post-marketing surveillance of patients with ALS for up to 5 years. Efficacy data will be compared with appropriate external control data.
Methods: Overall, more than 800 ALS patients who are naive pertaining to edaravone treatment have been enrolled and will be followed up for 5 years. Efficacy assessments: 1) Duration of survival and duration until invasive tracheal intubation up to 5 years; 2) Clinical events such as introduction of tube feeding, gastrostomy, and intermittent non-invasive ventilator assistance up to 1.5 years; 3) ALSFRS-R score up to 1.5 years. Safety assessments: Adverse events up to 1 year. The study was conducted in accordance with ministerial ordinance on Good Post-marketing Study Practice in Japan.
Results: The enrollment ended in October 12, 2017. In this poster, we will report the information on patient background and safety data collected on October 2018.
Discussion: The survey is proceeding as planned. These data may be useful not only for medical staff and ALS patients in Japan but also in Korea and the United States. This survey is expected to take a lead in the evaluation of drugs against neurological disorders.
Acknowledgments: We would like to thank patients and investigators for taking part in this post-marketing surveillance. p-value communications provided editorial support. The study

was funded and conducted by Mitsubishi Tanabe Pharma Corporation, Inc., Osaka, Japan (MTPC). KI, SY, MH, and HM are employees of MTPC.

2080: **Tideglusib, a drug candidate for the treatment of ALS**

Loreto Martinez-Gonzalez (1), Gracia Porras (1), Carmen Gil (1), Valle Palomo (1,2), Eva de Lago (2,3), Claudia Gónzalo Consuegra (3), Angeles Martín-Requero (1,2), Ana Martinez (1,2)
(1) Centro de Investigaciones Biológicas-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain.
(2) Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Spain.
(3) Instituto de Investigación en Neuroquímica, Dpto Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense de Madrid, Spain.

The discovery of an effective treatment for ALS is probably the most important challenge that all the ALS community need to achieve. Innovative drug discovery and drug repurposing may fulfill this goal in well oriented target-based programs.
TDP-43 is the main pathological proteinopathy present in ALS, being the main therapautic target of our research (1). We have implemented a multidisciplinary and coordinated program called ALS_Madrid with the main goal to discover small molecules able to recover the homeostasis of this nuclear protein. We used human-based cellular models (lymphoblasts from ALS patients) that are able to recapitulate the TDP-43 pathology (2), to show differences (if any) between sporadic and familiar ALS samples in response to the different new treatments.
As different protein kinases are involved in TDP-43 pathology, some protein kinases inhibitors may have an important role in ALS future therapy. That is the case of tideglusib, a GSK-3 inhibitor in clinical trials for autism and myotonic dystrophy with a wide safety window. One of the main goals of ALS_Madrid program is the repurposing of this drug for ALS based in different experimental facts that link GSK-3 with ALS in patients (3,4).
Here, we have shown how GSK-3 activity is increased in lymphoblast from sporadic ALS patients, with an increase in TDP-43 phosphorylation. Treatment with tideglusib decreased not only phospho-TDP-43 levels but also recover its nuclear localization. Moreover, this effect is also observed in vivo after oral administration for 5 weeks to a tg-TDP-43 (A315T) mouse model. Further data of this last study are under study.
Based on these recent data we may postulate tideglusib as a new potential therapy for ALS ready to start clinical trials phase II.

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2085: **Open-label extension trial for ALS participants who complete the Phase II, placebo-controlled CENTAUR trial**

Joshua Cohen(1), Justin Klee(1), Kent Leslie(1), Patrick Yeramian(1), Nazem Atassi(2), Sabrina Paganoni(2)
1 - Amylyx Pharmaceuticals, Cambridge, MA, USA
2 - Massachusetts General Hospital, Boston, MA, USA

Amylyx has developed a novel therapeutic, AMX0035, for the treatment of ALS. AMX0035 is a combination of two compounds, Sodium Phenylbutyrate (PB) and Tauroursodeoxycholic

Acid (TUDCA). AMX0035 is currently under investigation in the 24-week, parallel-group, double-blind, placebo-controlled CENTAUR trial.
In April 2018, Amylyx initiated the CENTAUR Open Label Extension Study (CENTAUR-OLE) designed to provide longer term access to AMX0035 for patients with Amyotrophic Lateral Sclerosis (ALS) who participated in the CENTAUR study. All patients who completed the randomized, double-blind AMX0035 study were eligible to participate in CENTAUR-OLE. The primary objective of the study is to assess long-term safety of oral (or feeding tube) administration of AMX0035 via sachet (3g PB and 1g TUDCA) twice daily. Over the extension period, patients will be evaluated for ALSFRS-R, isometric muscle strength (ATLIS), vital capacity, and disease-related outcomes, including gastric tube placement, use of permanent invasive ventilation, and hospitalizations.
Here, Amylyx reports the design of the CENTAUR-OLE and early observations, including the proportion of patients who elected to participate in the open-label study."

2086: **Clinical trial design for a Phase II, randomized, placebo-controlled trial of AMX0035 in Amyotrophic Lateral Sclerosis (CENTAUR)**

Joshua Cohen(1), Justin Klee(1), Kent Leslie(1), Patrick Yeramian(1), Nazem Atassi(2), Sabrina Paganoni(2)
1 - Amylyx Pharmaceuticals, Cambridge, MA, USA
2 - Massachusetts General Hospital, Cambridge, MA, USA

Amylyx has developed a novel therapeutic, AMX0035, for the treatment of ALS. AMX0035 is a combination of two compounds, Sodium Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA). PB and TUDCA have been individually tested in ALS clinical trials and showed promising early results, yet there has been no follow-up. Amylyx discovered a synergy between these two compounds when administered together at specific ratios across multiple preclinical models. In these models, AMX0035 blocked neuronal death and neuroinflammation through simultaneous inhibition of ER and mitochondrial stress. AMX0035 is being evaluated in a clinical trial in patients with ALS (CENTAUR). CENTAUR is a 24-week, parallel-group, double-blind, 2:1 randomized, placebo-controlled study. The trial completed enrollment in March 2019, with data expected November 2019.
Design considerations were made to power CENTAUR to detect changes in the slope of ALSFRS-R over 24 weeks. Analysis of the PRO-ACT database found that patients <540 days from symptom onset with a EEC diagnosis of Definite ALS experience considerably faster disease progression than the overall ALS patient population. Applying these criteria to a controlled, independent patient cohort (the ceftriaxone clinical trial) provided the sample size calculation basis for CENTAUR. Participants will be evaluated every six weeks for functional measures, muscle strength, and biomarkers. Muscle strength will be measured by ATLIS (Accurate Test of Limb Isometric Strength), which was shown to more sensitively detect disease progression than the ALSFRS-R. Biomarkers will include plasma levels of neurofilament light and heavy chains, and SUVr of the TSPO PET ligand PBR28. Neurofilaments will be used as a measure of axonal degeneration and will be measured longitudinally to assess potential correlations with clinical outcomes. A sub-study will evaluate TSPO uptake to assay drug effects on neuroinflammation.

1966: **ALS-RAP: The reproducible antibody platform initiative for ALS research**

Carl Laflamme(1), Tracy Keates(2), Carolyn Marks(3), Rina Shlaifer(1), Thomas M Durcan(1), Susanne Gräslund(3), Peter S. McPherson(1), Opher Gileadi(2)*.
1. Montreal Neurological Institute, McGill University, Montreal, Canada.
2. SGC, University of Oxford, Oxford, UK.
3. SGC, Karolinska Institute, Stockholm, Sweden.

Background: High-quality antibodies are crucial reagents in biomedical investigation. Anecdotal evidence suggests that approximately 50% of commercially-available antibodies are not fit for purpose (1). In particular, antibodies may not be specific to the target protein; may not be useful in the chosen application (western blot, immunocytochemistry, immunoprecipitation) when detecting native levels of expression; or may suffer batch variation (especially polyclonal antibodies).
Objectives: Three amyotrophic lateral sclerosis (ALS) charities, the ALS association (US), Motor Neurone Disease Association (UK) and the ALS Society of Canada, have jointly funded the ALS-RAP partnership. This project joins researchers in the UK, Canada and Sweden to generate thoroughly characterized recombinant antibodies to a selected list of proteins involved in ALS. The validation methods and the data for each antibody will be shared openly with the community, and all antibodies will be available from vendors.
Methods: The key elements of the antibody generation and characterization pipeline are (2-3):
(1) Folded proteins or protein domains used as antigens (4).
(2) Antibodies are selected from recombinant libraries using phage display.
(3) Alternative antibody sources will be incorporated, but all should yield fully-sequenced recombinant binders.
(4) Validation experiments are based on cell lines exhibiting physiological levels of target protein expression paired with isogenic knockout cells.
(5) Validation includes immunoprecipitation and mass spectrometry (IP/MS) as well as western blotting and immunostaining from the paired cell lysates.
Results: (1) We have tested our validation procedure on panels of commercial antibodies to the well-studied ALS protein C9ORF72. We identified a single monoclonal antibody specific for western blot and a second monoclonal antibody effective for immunoprecipitation, whereas all other antibodies tested (14 in total) were non-specific. No antibodies appeared specific for immunofluorescence. Unfortunately, several of the disqualified antibodies have already been used in published work.
(2) We present the current pipeline of purified antigens and antibody screens.
Conclusions:
(1) Currently available antibodies are not rigorously tested, limiting the validity of the resulting research data.
(2) The ALS-RAP partnership is poised to provide high-quality antibodies for reproducible research.

1999: **Treatment experience with Riluzole oral suspension**

Lizio A(1), Gerardi F(1), Sansone VA(1,2), Lunetta C(1)*
(1) NEuroMuscular Omnicentre, Fondazione Serena Onlus, Milan, Italy.
(2) Dept. Biomedical Sciences of Health, University of Milan.

Background: Riluzole is the first drug licensed for treating ALS, with the indication to extend life or the time to mechanical ventilation. For many years, riluzole was only available as an oral tablet dosage form. Recently, an oral suspension (OS) of riluzole was made available.
Our aim was to evaluate disease progression and survival of a cohort of ALS patients treated with OS form compared to a cohort of patients treated with tablet (TB) and a cohort of untreated patients (UT).
Patients and Methods: We enrolled all ALS patients referred at NeMO clinical centre with a OS riluzole prescription from December 2014 up to December 2017. UT and TB ALS patients were retrospectively selected matching for age at onset and sex, with a ratio of 1:2:1

for UT, TB and OS patients, respectively. For each patient, we collected a set of demographic and clinical data at baseline and after a one year follow up period.
Results: We included 18 UT patients, 36 TB patients and 18 OS patients, with a median age ranging from 60 to 68 years across groups. At baseline, OS groups reported a significant lower median ALSFRS-r total score in comparison to TB and UT groups. All three groups reported significant intragroup differences in ALSFRS-r total score between baseline and the end of the follow-up period. At the end of the follow-up period, no significant difference was found in terms of ALSFRS-r total score among the three groups. However, after taking into account the median monthly decline of the ALSFRS-r total score calculated between onset and the end of the follow-up, OS group reported a significant slower decline of the ALSFRS-r total score in comparison to TB group.
No significant differences were found regarding survival among the three groups.
Conclusions: This study in three small cohorts showed a reduction in the disease progression rate in patients treated with the OS compared to patients treated with the TB form or to patients not treated with riluzole. However, due to the low number of patients we did not find any effect on survival. Further studies with larger cohorts are necessary to better define if this new formulation could improve the effect of riluzole in ALS patients.

2036: **Radicava® (edaravone) for Amyotrophic Lateral Sclerosis: Oral Formulation and Its Development Plan**.

Koji Takei (1), Tomoyuki Omura (1), Yoshinobu Nakamaru (1), Masae Kakubari (1), Tomoko Natori (1), Youichi Shiide (1), Yukiko Nishimura (1), Makoto Akimoto (1), Manabu Hirai (1), Joseph Palumbo (1)(2)
1. Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan.
2. Mitsubishi Tanabe Pharma Development America, Inc., Jersey City, NJ, USA.

Background: An intravenous (IV) formulation of edaravone (Radicut®/Radicava®) has been approved for use in amyotrophic lateral sclerosis (ALS) in Japan, South Korea, the United States, Canada, and Switzerland. An oral formulation of edaravone is being developed with an understanding of the specific needs of individuals with ALS who may experience difficulties with swallowing.
Objective: To assess pharmacokinetic (PK) parameters of a new oral formulation of edaravone and discuss its anticipated development.
Methods: A Phase 1, dose-ranging PK study was conducted in healthy volunteers.
Results: A single oral dose of approximately 100 mg of edaravone appeared to deliver Cmax and AUC exposure comparable to that of the approved 60 mg/60 min IV infusion. Caucasian subjects showed a PK profile similar to that of Japanese subjects. No new safety findings were observed following single doses of up to 300 mg of oral edaravone relative to IV infusion.
A clinical development plan for the oral suspension is ongoing. A PK bridging strategy is under consideration, including a pivotal study demonstrating equivalent PK between the oral and IV formulations. Additionally, a planned dose-ranging study is expected to investigate 3 different regimens for the oral formulation: an oral 4-week cycle (similar to the approved 2-week on/off IV dosing in ALS), daily oral dosing at the equivalent IV dose, and daily "high-dose" treatment, in partial fulfillment of US FDA postmarketing commitments for the approved IV formulation.
Conclusions: PK data has indicated that an oral formulation (~100 mg) of edaravone produces a profile similar to that of the current IV formulation. The oral formulation was well tolerated at doses up to 300 mg. The clinical development plan may help to provide the necessary data in support of registration for marketing authorization pending further discussion with health authorities.

Acknowledgments: p-value communications provided editorial support. The study was funded and conducted by Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan (MTPC). All authors are employees of MTPC. JP is also an employee of Mitsubishi Tanabe Pharma Development America, Inc., Jersey City, NJ, USA.

2088: **Specific biosensors in Drug Discovery For Amyotrophic Lateral Sclerosis.**

Carlota Tosat-Bitrián(1), Ana Martinez(1,2), Valle Palomo (1,2)*
(1)Structural and Chemical Biology, Centro de Investigaciones Biológicas-CSIC, C/Ramiro de Maeztu 9, 28040, Madrid, Spain.
(2) Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Spain.

Neurodegenerative diseases (NDs) constitute a major health, economic and social issue worldwide. Despite the efforts for understanding NDs there is a lack of knowledge of their molecular pathology which is crucial for developing new efficient treatments. Amyotrophic lateral sclerosis (ALS) is a ND characterized by motorneuron (MN) death that yield in progressive paralysis. The mechanism underlying selective MN death remains an essential question, becoming a critical target for drug development.[1]
Molecular profiling is an innovative powerful technology for unravel complex molecular pathways that underlie physiological and pathological processes. Quantum dots (QDs) are very promising tools to detect molecular mechanisms at the subcellular level as their properties are ideal for multiplexing applications. QDs are nanoparticles formed by CdSe core and a ZnS coating that offer unique photo luminescent properties. They present an improved photostability, large molar extinction coefficients, broad absorption and narrow emission spectra that enables improved detection sensitivity and multiplexed analysis.[2] QDs have efficiently been conjugated to monoclonal antibodies (mAB) forming QD-AB probes that can be used in immunofluorescence, implementing a multiplexing QD based immunoassay.[3] Using this technology, different key proteins in ALS like TDP-43, p-TDP-43, CK1 will be analyzed at the single-cell level in human cell models such as lymphoblasts and MN derived from induced pluripotent stem cells (iPSCs) from ALS patients.
The scientific aim of this project is to further contribute to the molecular unravelling of ALS, finding patterns in patients, screening for biomolecular targets of these diseases; and to explore molecular changes in key protein targets upon pharmacological treatment to help select therapeutic candidates.
QD-SpA. b) Shematic illustration of the technology. c) Parallel multiplexed staining with five pre-assembled QD-SpA-Ab probes.[3]

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Oral Communications

Session 1: Genomics.

2084: **microRNA genetic variants in human ALS**.

Chen Eitan (1*), Tsviya Olender (1), Elad Barkan (2), Rick van der Spek (3), Sara Pulit (3), Elik Chapnik (1), Kristel R. van Eijk (3), Lindy Kool (3), Joke van Vugt (3), Sali M.K. Farhan (4), William Sproviero (5), Aleksey Shatunov (5), Ashley R Jones (5), Pamela J Shaw (6), Daphna Rotschild (2), Omer Weissbrod (2), Eran Segal (2), Christopher E Shaw (5), Leonard H. van den Berg (3), Ammar Al-Chalabi (5), John E. Landers (7), Robert H. Brown Jr (7), Jan H. Veldink (3), and Eran Hornstein (1) in collaboration with Project MinE ALS sequencing consortium.

1 Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

2 Department of Computer Science And Applied Math, Weizmann Institute of Science, Rehovot, Israel.

3 Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands.

4 Analytic and Translational Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Harvard Medical School and the Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Boston, MA, USA.

5 Department of Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK.

6 Department of Neuroscience, Sheffield Institute of Translational Neuroscience, University of Sheffield, Sheffield, UK.

7 Department of Neurology, University of Massachusetts Medical School, Worcester, MA.

Traditional human genetics focuses on variants that are deleterious for protein translation or function, but methods to explore the non-coding genome are not established. Here, we developed genetics methodologies for calling qualifying variants in non-coding regions, which enabled testing association with ALS in Project MinE's 4281 ALS patients and 1838 control genomes. We test hypotheses for variant burden in all known miRNA genes and in miRNA recognition elements (MREs) at all known 3' untranslated regions (3'UTRs). We demonstrate a highly significant signal, emerging form variants in the 3_UTR of Interleukin 18 Receptor Accessory Protein (IL18RAP). This association signal is comparable with the rare variant burden signal of the coding regions of known ALS genes, NEK1 and SOD1. Variants at IL18RAP 3'UTR seems protective, as they are prevalent in controls more than in ALS genomes. We independently assessed a large cohort of 62,784 non-ALS genomes from NHLBI's Trans-Omics for Precision Medicine (TOPMed), which further confirmed the robustness of the findings. Finally, we reveal that the mean age of onset in ALS patients harboring protective IL18RAP variants significantly delayed, by approximately 10 years, relative to the mean age of onset in patients that lack the IL18RAP protective variants. The work is innovative in interrogating non-protein coding genetic mechanisms by ALS whole-genome sequencing data and suggests intriguing neuroprotective immunomodulatory targets for therapy development.

1950: **Serum microRNA profiles reveal an involvement of FXR1 and FXR2 in amyotrophic lateral sclerosis.**

Axel Freischmidt(1)*, Anand Goswami(2), Katharina Limm(3), Vitaly L. Zimyanin(4), Maria Demestre(5), Karlheinz Holzmann(6), Anika M. Helferich(1), Priyanka Tripathi(2), Alfred Yamoah(2), Peter J. Oefner(3), Tobias M. Böckers(5), Eleonora Aronica(7), Dietmar R. Thal(8), Peter M. Andersen(9), Albert C. Ludolph(1), Andreas Hermann(4), Joachim Weis(2), Jörg Reinders(3), Jochen H. Weishaupt(1).

(1) Department of Neurology, Ulm University, Ulm, Germany.

(2) Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany.

(3) Institute of Functional Genomics, University of Regensburg, Regensburg, Germany.

(4) Department of Neurology, Technische Universität Dresden and Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Dresden, Germany; German Center for Neurodegenerative Diseases, Dresden Research Site, Dresden, Germany.
(5) Institute for Anatomy and Cell Biology, Ulm University, Ulm, Germany.
(6) Core Unit Genomics, Ulm University, Ulm, Germany.
(7) Amsterdam UMC, University of Amsterdam, Department of (Neuro)Pathology, Amsterdam Neuroscience, Amsterdam, the Netherlands.
(8) Department of Neuroscience, KU-Leuven and Department of Pathology, UZ-Leuven, Leuven, Belgium.
(9) Institute of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden.

Knowledge about converging disease mechanisms in the heterogeneous syndrome amyotrophic lateral sclerosis (ALS) is rare, but warranted for the development of therapies with efficacy for the majority of ALS patients. Previously, we identified a subset of serum microRNAs (miRNAs) downregulated in familial (fALS) and the majority (~60%) of sporadic ALS (sALS) patients, but also in pre-manifest mutation carriers. A common 5-nucleotide-sequence motif (GDCGG; D = G, A or U) was highly significantly enriched in these ALS-related miRNAs. We thus hypothesized the deregulation of one or several protein(s) binding specifically to this consensus motif is responsible for an altered abundance of the ALS-linked miRNAs. Using miRNA-pulldown assays followed by mass spectrometry and extensive biochemical validation, we found that all three members of the Fragile-X-protein family, FMR1, FXR1 and FXR2, directly and specifically interact with GDCGG-miRNAs through the RGG/RG-domains located in their disordered C-termini. In vitro binding studies on a transcriptome-wide scale confirmed preferential association of this protein family with ALS-related miRNAs. Furthermore, immunohistochemistry of patient lumbar spinal cord sections revealed aberrant expression levels and aggregation of both, FXR1 and FXR2, in fALS (C9ORF72- and FUS-linked) as well as sALS patients. Analyses of ALS autopsies and different iPSC-derived motoneurons with FUS mutation consistently showed co-aggregation of FXR1 with FUS. The aggregate staining pattern for FUS and FXR2 was less consistent but suggest a role also of FXR2 in the formation of FUS inclusions. Hence, our comprehensive approach was able to bridge the distance from blood miRNA changes to the discovery of novel neuropathological CNS markers, and suggests an involvement of the Fragile-X-related proteins in fALS and sALS. The findings may be the basis to uncover new disease mechanisms relevant to the majority of ALS patients, but also underscore the relevance of systemic, extra-CNS aspects of ALS.

2053: **C9orf72 intermediate expansions of 24–30 repeats are associated with ALS.**

Alfredo Iacoangeli (1,2)*, Ahmad Al Khleifat (2), Ashley R Jones (2), William Sproviero (2), Aleksey Shatunov (2), Sarah Opie-Martin(2), Karen E Morrison (3), Pamela J Shaw (4), Isabella Fogh(2,5), Christopher E Shaw(2,6), Richard J Dobson (1,7,8), Stephen J Newhouse(1,7,8), Ammar Al-Chalabi (2,9).
1)Department of Biostatistics and Health Informatics, King's College London, London, UK.
2)Maurice Wohl Clinical Neuroscience Institute, King's College London, Department of Basic and Clinical Neuroscience, London, UK.
3)Faculty of Medicine, University of Southampton, University Hospital Southampton, UK.
4)Sheffield Institute for Translational Neuroscience, University of Sheffield, UK.
5)Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy.
6)UK Dementia Research Institute, King's College London, UK.
7)Farr Institute of Health Informatics Research, UCL Institute of Health Informatics, University College London, London, UK;
8)National Institute for Health Research (NIHR) Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London London, UK.
9)King's College Hospital, Bessemer Road, London, SE5 9RS, UK.

A gigantic expansion of a hexanucleotide repeat GGGGCC in C9orf72 is the most common known cause of ALS accounting for ~40% of familial cases and ~7% of sporadic cases in the European population. In most people, the repeat length is 2, but in people with ALS, hundreds to thousands of repeats may be observed. Currently, 30 repeats are widely considered to be the threshold above which an expansion is classified as causative. A small proportion of people have an intermediate expansion, of the order of 24 to 30 repeats in size, and it remains unknown whether intermediate expansions confer risk of ALS in the same way that massive expansions do. Previous studies that tried to investigate the association of this intermediate repeat with ALS were limited by its rarity which resulted in insufficient statistical power and therefore inconclusive results. In this study we overcame this limitation by performing a meta-analysis of four previously published studies, for which data were available for repeats of size 24 or more, and our new British/ADNI cohort of 1,295 cases and 613 controls. The final dataset comprised 5,071 cases and 3,747 controls. Our meta-analysis showed the association between ALS and the intermediate C9orf72 repeat (random-effects model OR = 4.2, 95% CI = 1.23-14.35, p-value = 0.02). Furthermore, we showed a different frequency of the repeat between the northern and southern European populations (Fisher's exact test p-value = 5x10-3). This up-to-date figure obtained from a meta-analysis on 5,071 ALS patients and 3,747 controls from 5 populations (UK, USA, Spain, Italy and France) provides definitive evidence for the association between intermediate repeats and ALS (Fisher's exact test p-value 2x10-4) and expands the role of C9orf72 in the development of ALS by enlarging the number of cases that can be explained, with direct implications for clinical practice. However, due to the very low frequency of intermediate repeats, we could not identify an exact cut-off for the number of repeats to distinguish between neutral and pathological expansions. Further investigation and larger sample sizes are needed to achieve this. International initiatives such as Project MinE, whose aim is to collect genetic data (including the length of the C9orf72 repeat) of over 10,000 thousand people with ALS, will be crucial to this end. Interestingly, we also observed a few controls with very large expansions that are only rarely observed in non-affected individuals.

1929: **GWAS in ALS identifies novel loci and insight into the genetic architecture.**
Rick van der Spek on behalf of the Project MinE ALS GWAS Consortium.

We present the largest GWAS in ALS to date, containing 23,375 cases and 91,223 controls and almost 20 million QC passing variants. More than 100 individual cohorts were merged into strata based on genotyping platform. We applied a state-of-the-art logistic mixed model per stratum, which we then meta-analysed. This approach yields 14 independent genome-wide significant loci, including those near known ALS genes that harbor rare variants implicated in familial ALS (C9orf72, SOD1, TBK1, KIF5A) and an additional 4 novel loci. These results show that either GWAS is now able to identify rare-variant signals through synthetic associations or common variants in these loci additionally confer to ALS risk. Due to the unique combination with individual level whole genome sequencing data provided by Project MinE, we interrogated the rare-variant architecture of ALS at high resolution. On top of that we will present novel results on the population specific genetic architecture of ALS.

2049: **Opportunities and pitfalls in unravelling the role of DNA methylation in ALS: a large-scale collaborative effort within Project MinE.**
Paul Hop on behalf of the Project MinE ALS Sequencing Consortium
UMC Utrecht Brain Center, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands

Epigenetic mechanisms, including DNA methylation and histone modifications, may explain how genes and the environment interact and contribute to the onset and progression of ALS. DNA methylation is the best characterized epigenetic modification that stably influences gene expression and is influenced by environmental exposures, genetic variation and stochastic processes. To elucidate the role of DNA methylation in ALS pathogenesis we have generated methylomic data for 8,934 Project MinE blood samples collected across 13 countries (2:1 case/control ratio) using Illumina 450k and EPIC arrays. In addition, genetic and environmental/lifestyle data were collected for the majority of these samples and as such provides a unique integrated dataset to study the ALS methylome. Following thorough quality control, we are currently performing epigenome-wide association studies (EWAS) to identify DNA methylation patterns associated with ALS risk. Critical examination of preliminary results led to the discovery of a substantial number of spurious associations that do not reflect actual DNA methylation differences in the targeted regions. Instead, these associations reflect DNA methylation differences in the C9orf72 repeat expansion — the most common mutation in ALS — due to cross-hybridization, which is a technical artefact caused by probe sequences that map to multiple regions. We discovered that up to 60% of the significant probes, that were intended to map to a specific location in the genome, mapped to the C9orf72 locus instead in samples with a C9orf72 repeat expansion. We therefore suggest a cautionary approach when interpreting results obtained with Illumina methylation arrays, especially now that they are increasingly being used in ALS research. We are currently developing an approach to detect spurious associations, due to cross-hybridization, that could otherwise lead to false inferences and misdirected follow-up research. Taking this into account, we aim to finish our EWAS studies in the near future, which we expect to provide novel insights into the role of DNA methylation in ALS.

1949: **Hexanucleotide repeat RNA-binding proteins as modifiers of RNA toxicity in C9orf72 ALS.**

Elke Braems (1,2), Valérie Bercier (1,2), Laura Fumagalli (1,2), Bart Swinnen (1,2,3), Philip Van Damme (1,2,3), Ludo Van Den Bosch (1,2).
(1) KU Leuven - University of Leuven, Department of Neurosciences, Experimental Neurology, Leuven, Belgium
(2) VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium
(3) University Hospitals Leuven, Department of Neurology, Leuven, Belgium.

The 'GGGGCC' repeat expansion in C9orf72 is the most frequent cause of ALS. Three disease-causing mechanisms have been proposed, including loss-of-function of C9orf72 and two gain-of-functions resulting from either sequestration of essential repeat RNA-binding proteins (RNA toxicity) or from the formation of toxic C9orf72 dipeptide repeat proteins (DPR toxicity). However, the individual contribution of each mechanism to the development of ALS has not been elucidated yet. In particular, RNA toxicity is not fully understood. In this study, a C9orf72 zebrafish model is used to identify and investigate modifiers of RNA toxicity starting from candidate repeat RNA-binding proteins, including important splicing factors and transcriptional regulators. Modifiers are assessed by overexpressing them via co-injection of mRNA encoding these candidate RNA-binding proteins together with RNA containing 90 'GGGGCC' repeats. Using this approach, Pur_, an RNA-binding protein, and p62, a key autophagy protein, have already been identified to prevent a repeat RNA-induced axonopathy. At present, four potential new modifiers have been discovered, with hnRNPK displaying the most prominent rescuing effect. Functional analysis of this protein uncovered two structural domains being essential for modifying RNA toxicity: NLS/KNS and RNA-binding domains (KH1-KH2-KH3). Hence, this suggests that both subcellular localization and RNA recognition play a role in the observed effect. Moreover, using a morpholino-based

approach, knockdown of hnRNPK results in aberrant branching and reduced axonal length, suggesting hnRNPK to be essential for axonal development. Furthermore, preliminary data display a cytoplasmic mislocalization of hnRNPK in patient iPSC-derived motor neurons, supporting previous results. These findings highlight hnRNPK as a promising new target for further research and validation in other established disease models, including C9orf72 patient iPSC-derived motor neurons, mouse models and post mortem tissue.

Session 2: Disease Mechanisms 1:

1955: **Transcriptional alterations induced by polyDPRs overexpression in ALS-neuronal cell model.**

Cristofani R. *(1), Grilli (2), Licata N.V. (3), Crippa V. (1), Cicardi M.E.(4), Rusmini P.(1), Tedesco B.(1) ,Ferrari V. (1,), Casarotto E. (1,), Galbiati M. (1), Carra S(2), Provenzani A(3), Poletti A.(1).
1. Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milano, Italy.
2. Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università degli Studi di Modena e Reggio Emilia, Modena, Italy
3. Laboratory of Experimental Neurobiology, Department of Cellular, Computational and Integrative Biology (CIBIO), Università degli Studi di Trento, Trento, Italy
4. Department of Neuroscience, Sidney Kimmel Medical College, Jefferson University Philadelphia, USA.

Familial and sporadic Amyotrophic Lateral Sclerosis (ALS) forms are associated with several mutated proteins such as: mutant SOD1, TDP-43, FUS, VCP, OPTN and C9ORF72. RAN (Repeat Associated Non-AUG) translation has been originally observed with CAG triplet repeat sequences, but it is activated also in the presence of the (G4C2)n stretch of the C9ORF72 gene, which give rise to RAN translated poly di-peptide repeats (DPRs).
Similarly to polyQ containing proteins, DPRs misfold, aggregate in the cytoplasm or nuclei of motor neurons and this is thought to lead to proteotoxicity. The protein quality control (PQC) system maintains protein homeostasis and counteract proteotoxicity, promoting the re-folding (via chaperones) or by degradation (via autophagy or proteasome) of misfolded proteins. In particular, chaperone assisted selective autophagy (CASA), which is mediated by the HSPB8-BAG3-HSP70 complex, targets misfolded proteins for degradation, including DPRs. We demonstrated that, boosting CASA via HSPB8 overexpression prevents DPR aggregation and mediated toxicity.
To investigate whether DPR and PolyGln peptides share, if any, pathophysiological mechanisms, we developed a novel cell model in which ATG dependent DPRs (GA, GP, GR, PA, PR) and PolyQ peptide are under the control of the inducible promoter Tet-On in the human neuronal cell line SH-SY5Y. We first evaluated DPRs and polyQ expression levels and induced toxicity. In our system, doxycycline induced lower expression of all DPRs mRNA copies, than polyQ mRNA copies. This is also recapitulated in toxicity assays, where cells expressing polyQ peptides, but not DPRs, show marked cell death. We sequenced the entire transcriptome of each cell line after induction of DPRs or polyQ production and performed a differential gene expression profile and an overrepresentation of the most relevant functional categories. Most relevant differences were found in cells expressing polyGR and polyPR, the two most highly aggregation prone DPRs, in which components of mitophagy and protein metabolism were found modulated.
Collectively, these data show that alteration of gene expression by aggregating prone DPRs is an early event occurring prior to toxicity.
GRANTS: FONDAZIONE TELETHON; FONDAZIONE CARIPLO; FRRB, PRIN, FONDAZIONE ARISLA; Joint Programme Neurodegenerative Disease and Kennedy's disease association.

1946: **Heterozygous Tbk1 loss has opposing effects in early and late stages of ALS in mice.**

David Brenner* (1), Kirsten Sieverding (1), Clara Bruno (1), Patrick Lüningschrör (2), Eva Buck (1), Simon Mungwa (2), Lena Fischer (1), Sarah J. Brockmann (1), Johannes Ulmer (1), Corinna Bliederhäuser (1), Clémentine E. Philibert (4), Takashi Satoh (3), Shizuo Akira (3), Séverine Boillée (4), Benjamin Mayer (5), Michael Sendtner (2), Albert C. Ludolph (1), Karin M. Danzer (1), Christian S. Lobsiger (4), Axel Freischmidt (1), and Jochen H. Weishaupt (1).
(1) Department of Neurology, University of Ulm, Ulm, Germany.
(2) Institute of Clinical Neurobiology, University Hospital of Wuerzburg, Wuerzburg, Germany.
(3) Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.
(4) Institut du Cerveau et de la Moelle Épinière, Institut National de la Santé et de la Recherche Médicale Unité 1127, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7225, Sorbonne Université, Paris, France.
(5) Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany.

Heterozygous loss-of-function mutations of TANK-binding kinase 1 (TBK1) cause familial ALS, yet downstream mechanisms of TBK1 mutations remained elusive. TBK1 is a pleiotropic kinase involved in the regulation of selective autophagy and inflammation. We show that heterozygous Tbk1 deletion alone does not lead to signs of motoneuron degeneration or disturbed autophagy in mice during a 200-d observation period. Surprisingly, however, hemizygous deletion of Tbk1 inversely modulates early and late disease phases in mice additionally overexpressing ALS-linked SOD1G93A, which represents a "second hit" that induces both neuroinflammation and proteostatic dysregulation. At the early stage, heterozygous Tbk1 deletion impairs autophagy in motoneurons and prepones both the clinical onset and muscular denervation in SOD1G93A/Tbk1+/_ mice. At the late disease stage, however, it significantly alleviates microglial neuroinflammation, decelerates disease progression, and extends survival. Our results indicate a profound effect of TBK1 on brain inflammatory cells under proinflammatory conditions and point to a complex, two-edged role of TBK1 in SOD1-linked ALS.

1974: **Loss of C9orf72 alters the immunophenotype but does not change the motor neuron loss of SOD1G93A mice.**

Francesca Sironi, Mattia Freschi, Giulia De Giovanetti, Jessica Cassarà, Valentina Herrera, Massimo Tortarolo, Caterina Bendotti*.
Molecular Neurobiology Lab, Department of Neuroscience, IRCCS - Mario Negri Institute, Milano, Italy.

The hexanucleotide repeat expansion in the C9orf72 gene (C9) is the most common genetic cause of ALS. In addition to the toxicity of the expanded repeat, the reduction in C9 transcript and protein levels in patient cells and brain tissue suggests that loss of function mechanisms may contribute to ALS. Recently, it has been shown that loss of C9 leads to neurodegeneration in human motor neurons (MNs) and hypersensitizes cells to stress. However, C9orf72 knockout (C9-KO) mice do not display overt neurodegenerative phenotype suggesting that both the loss and the gain of function mechanisms are required for MN pathology. Conversely, C9-KO mice develop an altered immune response with reduced autophagic microglial function and increased inflammatory state, mechanisms involved in ALS progression. Thus, to determine whether a loss of function of C9 may have an impact on the course of the disease, in this study we investigated how the C9 gene deletion influences the mouse immune-phenotype both systemically and in the spinal cord and whether it could have a modulatory effect on the disease progression of SOD1G93A mice. To achieve this, we developed a constitutive C9-KO mouse line on C57Bl/6 genetic background, which was then crossbred with SOD1G93A mice. C9-KO mice showed splenomegaly associated with increased levels of lymphocytes CD4+ and CD8+ and reduced Treg in spleen and spinal cord. No motor dysfunction neither spinal MN loss was observed

in C9-KO mice at 6 months of age, however, slight but significant atrophy and partial denervation were found in tibialis anterior muscle. In SOD1G93A mice, neither the impairment of paw grip strength nor the spinal MN loss was influenced by the C9 deletion. However, the body weight loss and hind limb muscle atrophy of SOD1G93A mice were reduced in C9 depleted mice while the denervation of the neuromuscular junction was increased, suggesting an enhanced neuromuscular dysfunction in SOD1G93A/C9-KO animals. Despite the loss of spinal motor neurons was unchanged in SOD1G93A/C9-KO mice, the reactive microglia and the T cells infiltrate were significantly reduced in these mice compared to the SOD1G93A mice and this correlated with the reduced levels of proinflammatory cytokines. This suggests that even if the constitutive loss of C9 attenuates the inflammatory environment around the MNs, this phenomenon does not affect the neuronal toxicity induced by mutant SOD1. Supported by TRANS-ALS- Regione Lombardia (no. 2015–0023).

2097: **Gain and loss of function synergize in the C9ORF72 HRE pathogenicity**.

Hortense de Calbiac(1)*, Maria-Letizia Campanari(1), Doris Lou Demy(2), Anca Marian(1), Sorana Ciura(1), Edor Kabashi(1).
1 Imagine Institute, Paris, France
2 Pasteur Institute, Paris, France.

The most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) is the hexanucleotide repeat expansion (HRE) in C9ORF72. Proposed mechanisms for C9ORF72 HRE pathogenicity include loss-of-function of C9ORF72, as well as gain-of-function caused by dipeptide repeats (DPRs) translated from the HRE.
We developed a zebrafish model that combines both gain and loss-of-function properties by the concomitant expression of poly(GP) and knock-down of the zebrafish orthologue of c9orf72. We demonstrate that loss-of-function of c9orf72 is essential to trigger poly(GP) accumulation, resulting in motor neuron degeneration and paralysis. Both these motor features can be prevented by caspase-9 inhibition. In addition, autophagy induction with rapamycin treatment mitigates the motor deficit due to the c9orf72 synergistic loss-of-function and poly(GP) toxicity. Knock-down of the autophagy receptor sqstm1, which is also mutated in some ALS/FTLD patients, also aggravated the toxicity of poly(GP).
These results indicate that gain and loss-of-function act in common pathogenic mechanisms to synergize the C9ORF72 pathogenicity, and need to be addressed to treat ALS and FTLD.

1982: **TDP-43 peptide seeding in the context of aggregation and phase separation.**

Donya Pakravan(1)*, Emiel Michiels(2), Anna Bratek-Skicki(3), Peter Tompa(3), Ludo Van Den Bosch(1)
1 VIB, Center for Brain & Disease, Laboratory of Neurobiology, B-3000 Leuven, Belgium.
2 VIB-KU Leuven Center for Brain & Disease Research O&N 4, 6e verd Campus Gasthuisberg Herestraat 49, Leuven, Belgium
3. VIB-VUB Center for Structural Biology Building E Pleinlaan 21050 Brussels, Belgium.

Transactive response DNA-binding protein (TDP-43) is a predominantly nuclear protein with roles in regulating RNA transcription, splicing, miRNA production and transport. In disease, TDP-43 accumulates in the cytoplasm in almost all amyotrophic lateral sclerosis (ALS) and many frontotemporal lobar dementia (FTLD) cases. Many of the TDP-43 aggregates found in ALS patient spinal cord and brain samples are ubiquitinated and hyperphosphorylated, and the low complexity domains (LCDs) of have been associated with the formation of these amyloid-like fibrillar aggregates. Despite preliminary observations

linking liquid-liquid phase separation and protein aggregation, little is known about the role of protein seeding in the phenomenon of droplet formation and maturation. TDP-43 oligomers have recently been proven to transfer in a neuron-to-neuron manner indicating this seeding mechanism we also know TDP-43 is found within these membraneless stress granule components. Does the process of peptide seeding influence phase separation or does it independently self-template further aggregation? To utilize a biophysical approach to ascertain the changes in protein turbidity we found core peptide residues within the LCD which influence the aggregation behavior of the protein. In addition, we checked how these residues affected protein dynamics. As such, we hope that by studying seeding mechanisms we can finally understand the relationship between phase separation and aggregation.

1909: **Cognitive deficits in ALS are a marker of localized TDP-43 cerebral pathology.**

*Jenna M. Gregory (1,2), Karina McDade (1,2), Thomas Bak (2,3), Suvankar Pal (1,2), Siddharthan Chandran (1,2), Colin Smith (1,2) and Sharon Abrahams (2,3).
(1) Centre for Clinical Brain Sciences, University of Edinburgh, Chancellor's Building, Edinburgh, EH16 4SB, UK.
(2) Euan MacDonald Centre for Motor Neurone Disease Research, University of Edinburgh, Chancellor's Building, Edinburgh, EH16 4SB, UK.
(3) Human Cognitive Neuroscience, Psychology, 7 George Square, University of Edinburgh, EH8 9JZ, UK.

Objectives: ~35% of amyotrophic lateral sclerosis (ALS) patients exhibit mild cognitive deficits in, executive functions, language and fluency, without dementia. The precise pathology of these extra-motor symptoms has remained unknown. This study aimed to determine whether (i) cognitive impairment as detected by the Edinburgh Cognitive ALS Screen (ECAS) was a good in vivo predictor of TDP-43 pathology and (ii) the cognitive profile would relate to specific regional distribution of pathology in the frontal and temporal lobes.
Methods: In-depth neuropathological analysis of 27 non-demented ALS patients who had undergone cognitive testing (ECAS) during life. Analysis involved assessing TDP-43 accumulation in brain regions specifically involved in executive function, language function and fluency to ascertain whether functional deficits would relate to a specific regional distribution of pathology.
Results: All patients with cognitive impairment had TDP-43 pathology in extra-motor brain regions (positive predictive value of 100%). The ECAS also predicted TDP-43 pathology with 100% specificity in brain regions associated with executive function, language and fluency domains. We also detected a subgroup with no cognitive dysfunction, despite having substantial TDP-43 pathology, so called mismatch cases.
Interpretation: Cognitive impairment as detected by the ECAS is a valid predictor of TDP-43 pathology in non-demented ALS. The profile of mild cognitive deficits specifically predicts regional cerebral involvement. These findings highlight the utility of the ECAS, in accurately assessing the pathological burden of disease.

Session 3: Clinical and Epidemiology:

2021: **Occupations, socio-economic position, and the risk of amyotrophic lateral sclerosis. The Euro-MOTOR study**.

Fabrizio D'Ovidio(1*), Anne E Visser(2), Angelo d'Errico(3), Ettore Beghi(4), Giancarlo Logroscino(5), Roel CH Vermeulen(6), Jan H Veldink(2), Leonard H van den Berg(2), Orla Hardiman(7), Adriano Chiò(1), for the Euro-MOTOR consortium.
1 "Rita Levi Montalcini" Department of Neurosciences, University of Turin, Turin, Italy.

2 Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands.
3 Department of Epidemiology, Local Health Piedmont Region, ASL TO3, Grugliasco (TO), Italy.
4 Department of Neurosciences, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.
5 Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari "Aldo Moro", Bari, Italy.
6 Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands.
7 Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

Introduction. Literature on the association between occupations and the risk of ALS is rich in contributions, but with inconsistent results and unclear definition of risk factors.
Methods. A population of 1,374 cases and 2,648, who reported at least one job carried out for a minimum of 3 years, were extracted from the international case-control study (Euro-MOTOR), performed in Ireland, The Netherlands and Italy, from 2011 to 2014. A complete occupational history was collected and classified using the 1988 International Standard Classification of Occupations, (IISCO-88), through which Socio-Economic Position (SEP) was assigned to the study population, based on both the Treiman's international prestige scale (SIOPS) and the European Socio-economic Classification (ESEC). Data were analysed using logistic regression models adjusted for gender, age, education, ALS Centre, alcohol, smoking habit, and leisure time activity.
Results: The risk of developing ALS appeared positively associated with manual jobs and negatively associated with non-manual jobs. Agriculture and fishery workers (HR=1.43), tailors, dressmakers and hatters (HR=1.76), and helpers and cleaners (HR=1.71) were the jobs at significantly higher ALS risk. On the contrary, life science and health professionals (HR=0.47) –nursing and midwifery professionals (HR=0.55) in particular –, life science technicians (HR=0.69), and secretaries (HR=0.73) displayed a lower risk.
However, analyses stratified by sex and country showed a more articulated picture of associations. Agriculture and fishery workers showed an increased risk especially in women (HR=2.17) and in Italy (HR=1.90). Waiters, waitresses services workers were found at higher risk in Italy (HR=2.32), as well as shop salespersons and demonstrators (HR=2.18). Craft and related trades workers had an increased risk especially in Italy (HR=1.81), and in the Netherlands (mostly driven by the extraction and building trades workers, HR=1.48).
The analysis on SEP reported associations along the same lines of the ISCO88 macro-groups of occupations, with trends of increased risk with decreased SEP for both the ESEC and the SIOPS scales.
Conclusion: The study showed increased risks of ALS for manual jobs, in particular for agriculture and fishing, and for lower SEP classes with clear trends. Findings appeared extremely different among countries and gender, sign of complex and variegated job-related factors which could affect ALS occurrence.

2052: **The D50 Progression Model for Amyotrophic Lateral Sclerosis: Extension to and Validation in Global Cohorts.**

Nayana Gaur (1), Benjamin Ilse (1), Torsten Grehl (2), Matthieu Moisse (3), Philip van Damme (3), Christoph Neuwirth (4), Markus Weber (4), Umberto Manera (5), Adriano Chio (5), Jan Veldink (6), Leonard van den Berg (6), Thomas Meyer (7), Blaz Koritnik (8), Janez Zidar (8), Paul Wicks (9), Philippe Corcia (10), Philippe Couratier (10), Marta Gromicho (11), Mamede de Carvalho (12), Katarzyna Szacka (13), Magdalena Ku_ma-Kozakiewicz (13), Frauke Fartmann (14), Susanne Petri (14), Hilmi Uysal (15), Matthew Kiernan (16), Julian Grosskreutz (1*).
1 Department of Neurology, Jena University Hospital, Jena, Germany
2 Department of Neurology, Alfred Krupp Krankenhaus Rüttenscheid, Essen, Germany.
3 University Hospitals Leuven, Department of Neurology, Leuven, Belgium.

4 Neuromuscular Disease Unit/ALS Clinic, Cantonal Hospital St. Gallen, Switzerland
5 Department of Neuroscience, University of Torino, Torino, Italy
6 Department of Neurology, University Medical Center Utrecht, Utrecht, The Netherlands
7 Center for ALS and other motor neuron disorders, Charité – Universitätsmedizin Berlin, Germany
8 Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia
9 PatientsLikeMe, Inc., Cambridge, MA, USA
10 Faculté de Médecine, Université François-Rabelais de Tours, Tours, France
11 Instituto de Medicina Molecular, Instituto de Fisiologia, Faculdade de Medicina, Universidade de Lisboa
12 Department of Neurosciences and Mental Health, Hospital de Santa Maria-CHLN, Lisbon, Portugal
13 Department of Neurology, Medical University of Warsaw, Warsaw, Poland
14 Department of Neurology, Hannover Medical School, Hannover, Germany
15 Department of Neurology, Akdeniz University Faculty of Medicine, Antalya, Turkey
16 Brain and Mind Center, The University of Sydney, School of Medicine, Sydney, Australia.

Background: The D50 model was developed to address drawbacks associated with the traditional ALSFRS-R-derived "Progression Rate" (PR); the latter presupposes that progression is linear and remains temporally fixed. However, progression (as measured by ALSFRS-R decay) is heterogenous and varies profoundly between and within individuals across time. The model uses individual functional loss profiles to describe patients independent of the time-point at which they are assessed.
Objectives: Having already conducted an initial validation using the PROACT database, we aimed to further test model applicability and utility within a demographically heterogenous cohort.
Methods: Iterative least-square fitting of all available ALSFRS-R scores was used to describe disease progression using a sigmoidal state transition from full health to complete functional loss. This yields 3 summative descriptors: D50 (time taken for ALSFRS-R score to reach 24), dx (time constant of ALSFRS-R decay), and relative D50 (describing individual disease covered in reference to D50, with 0=disease onset and 0.5=time-point of halved functionality. We also developed 2 local descriptors of disease activity: calculated functional loss & functional state.
Results: Summative disease descriptors were calculated for 16,871 patients from 14 centers using approximately 116,223 ALSFRS-R data points. All centres had a mean D50 < 50 months. D50 and dx correlated linearly in all centers. rD50 aligns individual patients in terms of elapsed time to 50% loss of function, thus allowing comparability despite different time scales for milestones e.g. spread to 2nd region averaged at rD50=0.15±0.15 with cFS of 43.47±5.09; diagnosis at rD50=0.21±0.13 with cFS of 41.05±5.50; spread to 3rd region at rD50=0.27±0.22 with cFS of 40.16±7.41; wheelchair at rD50=0.40±0.20 with cFS of 31.15±9.68; and death at rD50=0.69 with cFS of 18.88±10.46.
The model provides a useful supplement to conventional staging systems like King's and MITOS. Patients can be easily classified into mathematically-derived disease phases.
Conclusion: The model provides meaningful descriptors of overall disease aggressiveness, local disease activity, and a unified linear time scale to describe individual disease progression. It enables pseudo-longitudinal interpretations of cross-sectional data.
Acknowledgment: This work is supported by the BMBMBF in the framework of the JPND programme ONWebDUALS.

1997 : **Pulmonary Function and ALS Staging Systems.**

Philippe Couratier(1)*, Geraldine Lautrette (1), Marie Penoty (2), Stéphane Beltran (2), Salah Bakkouche (2), Philippe Corcia (2).
1: Centre de Référence SLA et autres maladies du neurone moteur CHU Limoges. Fédération Litorals.
2: Centre de Référence SLA et autres maladies du neurone moteur CHU Tours. Fédération Litorals.

Two staging systems have been recently validated in ALS, the King's College Staging System and the MiToS (Milan Torino Scale) functional staging system. Both systems have been shown to be complementary and allow a rapid and reliable functional assessments and comparison of treatment models. However, it remains unknown whether pulmonary function impacts or drives changes in ALS stages.
The objectives were to understand how pulmonary function is represented in each stage of the King's College Staging and MiToS Staging Systems and to determine the degree to which vital capacity (VC) correlates with survival for each stage of the two staging systems.
Patients and methods: The following variables were collected in a French cohort of ALS patients: Sex, date of birth, date of first symptoms, date of diagnosis, site of onset, ALSFRS-R, MiToS Staging System, King's College Staging System, date or death or tracheostomy, NIV indications, SVC, FVC, and familial versus sporadic ALS. Stages will be determined for each patient from time of diagnosis and every three months thereafter. SVC and FVC will also be recorded for each of these timepoints. Determination of stage were (assessed twice by two separate blinded investigators based on the patient's electronic record). Cox proportional hazard regression models adjusted for age, gender.
Results: 559 ALS patients (sex ratio M:F 1;2; mean age at diagnosis: 64.3 yrs; 32.7% of bulbar forms; mean duration of survival 13.4 ± 12.8 months) were included. Mitos and King's were correlated with SVC and FVC at first evaluation and during the evolution. According to SVC and FVC at baseline divided in 4 subclasses (100%), the probability of a King's stage changing was greatest than those of the Mitos between 3 and 6 months from baseline. Similar results were obtained when patients were classified stage 1 on King's staging at baseline.
Conclusion: VC at baseline is a promising prognostic factor for survival and is related to King's and Mitos staging. VC may be a surrogate of disease stage progression.

1964: **Endophenotypes in ALS: Cognitive and psychiatric assessment of unaffected relatives of ALS patients.**

Emmet Costello*(1,2), Marie Ryan (1), Caroline McHutchison (3), Sharon Abrahams (3), Niall Pender (2), Orla Hardiman (1).
1. Academic Unit of Neurology, Trinity Biomedical Science Institute, Trinity College Dublin, Dublin, Ireland.
2. Department of Psychology, Beaumont Hospital, Dublin, Ireland.
3. Department of Psychology, The University of Edinburgh, Edinburgh, Scotland.

Background: Recent evidence has suggested a link between Amyotrophic Lateral Sclerosis (ALS) and neuropsychiatric illness. However, the nature of this relationship has yet to be explored. We aim to carry out neuropsychological and neuropsychiatric assessment on unaffected first and second-degree relatives of ALS patients. In doing so we aim to uncover if an endophenotype (intermediate phenotype) of ALS/neuropsychiatric risk exists. If so, we can improve the power of our genetic studies and gain further insight into the gene-disease process.
Method: Thirty-seven ALS patients, 172 first and second degree relatives and 69 controls were recruited as part of a larger project on gene pleiotropy in ALS. Participants completed in depth neuropsychological assessment of executive functioning, memory, language, visuospatial ability and attention. Participants also completed a self-report questionnaire of neuropsychiatric illness.
Results: ALS relatives performed significantly worse than controls on executive and memory tasks ($p<.01$). Sub-group analysis revealed that these deficits were attributable to relatives of familial ALS (FALS) patients and not relatives of sporadic ALS (SALS) patients. Deficits were most significant on the Colour Word Interference Task (CWIT), Verbal Fluency and the Rey Auditory Verbal Learning Test (RAVLT). Neuropsychiatric

assessment found that ALS relatives scored highly on the Community Assessment of Psychic Experience (CAPE), a screening measure of psychosis risk. Analysis of personality traits revealed that ALS patients and FALS relatives scored significantly lower on the openness to experience trait.
Discussion: These findings provide preliminary evidence of an endophenotype in ALS, characterised by poorer executive/memory performance, higher risk of psychosis and lower ratings of the openness personality trait. This endophenotype appears to be most apparent in families with a strong history of ALS. Our results support previous findings of a link between ALS and neuropsychiatric illness, and puts forward an endophenotype which can explain this link. These finding, if replicated, can improve statistical power in gene discovery studies, and lead to an improved understanding of the extended genetic, neuropsychological and neuropsychiatric profile in ALS

2012 **Executive impairment among C9orf72 repeat expansion carriers: An under recognized phenomenon**.

Marie Ryan* (1, 2), Emmet Costello (1, 3), Mark A Doherty (4), Mark Heverin (1), Russell L McLaughlin (4), Niall Pender (3), Orla Hardiman (1, 2).
1. Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.
2. Department of Neurology, Beaumont Hospital, Dublin, Ireland.
3. Department of Psychology, Beaumont Hospital, Dublin, Ireland.
4. Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland.

Background: Executive function is among the most heritable of psychological traits. The pathogenic GGGGCC C9orf72 hexanucleotide repeat expansion is a well described cause of ALS-FTD and in C9orf72 associated FTD, executive dysfunction is almost universal. However, subtle executive changes in the C9orf72 positive ALS patient population and pre-symptomatic carriers are not well characterised. Only 10% of C9orf72 positive patients are described as cognitive in onset. With developments in anti-sense oligonucleotide technologies having the potential to change the therapeutic landscape for C9orf72 positive ALS, delineating the clinical phenotype in the earliest stages of disease onset becomes paramount.
Methods: DNA samples from 961 individuals registered with the Irish ALS Register and 172 of their neurologically normal relatives were screened for C9orf72 repeat expansion. Executive impairment was defined as impaired verbal fluency or impairment on two other non-overlapping measures of executive functions.
Results: 76 patients with ALS and 20 of their relatives were found to carry the repeat expansion. 26 C9orf72 positive patients had co-existing FTD. 18 C9orf72 positive patients and 8 pre-symptomatic carriers had evidence of abnormal executive functioning. Patients without executive dysfunction were younger than those with executive dysfunction (mean age onset: executive function normal 53.5, executive function impaired 59.1, FTD 62.5 p=0.002.) Executively abnormal pre-symptomatic carriers were older than those who with normal executive function (mean age 55.6 v 36.5, p=0.001).
Conclusion: Cognitive onset disease is under recognized among C9orf72 positive ALS patients. The vast majority of C9orf72 positive ALS patients have executive dysfunction on presentation, along with 40% of pre-symptomatic carriers, suggesting degeneration in intrinsic networks associated with executive function arises early in the disease course. Nonetheless, age-dependent penetrance of the repeat expansion means younger patients carrying the expansion are less likely to manifest executive dysfunction on presentation. Serial neuropsychological testing is warranted to assess for developing cognitive and behavioural changes, particularly in younger patients.

Session 4: Disease Models:

1993: **Metabo-lipidomics of fibroblasts from ALS patients show alterations in purine, pyrimidine, energetic, and phospholipid metabolisms.**

Charlotte Veyrat-Durebex (1,2,4,7*), Céline Bris (1,2), Philippe Codron (2,3), Cinzia Bocca (2), Stéphanie Chupin (1), Philippe Corcia (4,5,6), Patrick Vourc'h (4,7), Rudolf Hergesheimer (4), Julien Cassereau (2,3), Benoit Funalot (6), Christian R Andres (4,7), Guy Lenaers (2), Philippe Couratier (6), Pascal Reynier (1,2), Hélène Blasco (2,4,7).
1. Département de Biochimie et Génétique, CHU d'Angers, Angers, France.
2. Unité Mixte de Recherche MITOVASC, CNRS 6015-INSERM 1083, Université d'Angers, Angers, France.
3. Centre de Ressources et de Compétences SLA, Service de Neurologie, CHU Angers, Angers, France.
4. Université de Tours, Inserm U1253, Tours, France.
5. Centre de Référence SLA, Service de Neurologie, CHRU Bretonneau, Tours, France.
6. Fédération des CRCSLA Tours et Limoges, LITORALS, France.
7. Laboratoire de Biochimie, CHRU de Tours, Tours, France.

Amyotrophic Lateral Sclerosis (ALS) is characterized by a wide metabolic remodeling, as shown by recent metabolomics and lipidomics studies performed in samples from patient cohorts and experimental animal models.
Here, we explored the metabolome and lipidome of fibroblasts from sporadic ALS patients (n=13) comparatively to age- and sex-matched controls (n=11), and the subcellular fraction containing the mitochondria and endoplasmic reticulum (mito-ER), given that mitochondrial dysfunctions and ER stress are important features of ALS patho-mechanisms. We also assessed the mitochondrial oxidative respiration and the mitochondrial genomic (mtDNA) sequence, although without yielding significant differences.
Compared to controls, ALS fibroblasts did not exhibit a mitochondrial respiration defect nor an increased proportion of mitochondrial DNA mutations. In addition, non-targeted metabolomics and lipidomics analyses identified 124 and 127 metabolites, and 328 and 220 lipids in whole cells and the mito-ER fractions, respectively, along with Partial Least-Squares – Discriminant Analysis (PLS-DA) models being systematically highly predictive of the disease. The most discriminant metabolomic features were the alteration of purine, pyrimidine and energetic metabolisms, suggestive of oxidative stress and of pro-inflammatory status. The most important lipidomic feature in the mito-ER fraction was the disturbance of phosphatidylcholine PC(36:4p) levels, which we had previously reported in the cerebrospinal fluid of ALS patients and in the brain from an ALS mouse model.
Thus, our results reveal that fibroblasts from sporadic ALS patients share common metabolic remodeling, consistent with other metabolic studies performed in ALS, opening perspectives for further exploration in this cellular model in ALS.

1983: **C9orf72 and TDP-43 iPS-derived motor neurons have alterations in calcium buffering**.

Ruxandra Dafinca(1)*, Nidaa Ababneh(1), Ana Candalija (1), Jakub Scaber(1), Paola Barbagallo(1), Lucy Farrimond(1), Kevin Talbot(1).
1. Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom.

Hexanucleotide expansions in the C9orf72 are the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), accounting for up to 50% of familial ALS cases. While mutations in TARDBP are a rare cause of ALS, the deposition of TDP-43 positive cytoplasmic inclusions remains a common neuropathology for approximately 97% of ALS cases, including C9orf72 cases. Identifying common pathways

between C9orf72 and TDP-43 would significantly contribute to our understanding of the disease mechanism.
The aim of this study is to identify which pathways of calcium homeostasis are dysregulated in C9orf72 and TDP-43 ALS/FTD cases and whether a common pathomechanism can be identified between the two mutations using patient iPS-derived MNs.
Methods: In this study, we differentiated patient motor neurons derived from induced pluripotent stem cells (iPSCs) carrying hexanucleotide expansions in the C9orf72 gene or mutations in TDP-43 (M337V and I383T). We generated isogenic iPSC lines where the expansions were successfully removed by CRISPR/Cas9 in C9orf72 iPSCs. Recording of calcium waves was performed under continuous perfusion of stimuli (50 mM KCl, 100 uM glutamate, 50 uM carbachol) or direct application of 10 uM thapsigargin.
Results and conclusions
In C9orf72 iPS-derived MNs we found significantly higher calcium release when neurons were depolarized. Clearance of calcium from the cytosol was significantly delayed in C9orf72 MNs after stimulation with KCl and glutamate, which correlated with low levels of the calcium-buffering protein calbindin and increased cell death. The levels of calcium binding proteins in TDP-43 MNs were not significantly different. An impairment was also observed in the calcium-buffering capacity of mitochondria in C9orf72 MNs, where low levels of the mitochondrial calcium uniporter (MCU) were detected and correlated with reduced uptake of Ca2+ from the cytosol compared to healthy controls and corrected MNs. The mitochondrial potential was reduced in C9orf72 MNs, while the TDP-43 MNs did not show differences when compared to healthy controls. In TDP-43 MNs, we found deficiencies in ER calcium release through the IP3 receptor when neurons were stimulated by carbachol, which correlated with ER stress. Both C9orf72 and TDP-43 MNs show upregulation of Ca2+ permeable glutamate receptor subunits, as well as an imbalance in the expression of mitochondrial Ca2+ uptake regulators.

1925: **Using patient-derived astrocytes to unravel the nuclear role of SOD1 and its link with nucleus/cytoplasm shuttling.**

Noemi Gatto (*1), Monika Myszczynska (1), Lydia Castelli (1), Guillaume Hautbergue (1), Pamela J Shaw (1), Laura Ferraiuolo (1).
1. Sheffield Institute for Translational Neuroscience, University of Sheffield (SITraN) , Sheffield , UK.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder and little progress has yet been made on the aetiology of the 90% of sporadic ALS (sALS) cases. The disease has been partly understood through studies focusing on the first gene associated with familial ALS (fALS), the gene encoding for superoxide dismutase 1 (SOD1). Mutations in SOD1, responsible for about 20% of fALS, were first identified in 1993.
Several pieces of evidence indicate that misfolded wild-type SOD1 may also be pathogenic in sporadic ALS (sALS), through different processes, such as misfolding, aggregation and prion-like pathogenic behaviour. Moreover, there is new evidence indicating that SOD1 could function as a transcription factor, responding to oxidative stress and its misfolding could affect gene transcription.
To study the effect of wild-type SOD1 in familial and sporadic ALS we have generated induced astrocytes from control and patient skin fibroblasts, through direct conversion. We have focused on astrocytes, as it has been reported that they actively contribute to motor neurone death in ALS and in a previous study SOD1 knock-down successfully reduced astrocyte toxicity.
Of relevance in this context, staining for misfolded SOD1, using the B8H10 antibody, showed a nuclear aggregation of the protein in sALS patient astrocytes. This poses a question on the role of SOD1 in this cellular compartment and potentially indicates that the

transcription profile of these cells might be altered as a consequence. SOD1 presence in the nucleus of patient iAstrocytes was confirmed through cell fractionation, thus indicating that SOD1 might indeed act as a transcription factor or a co-activator with important implications in case of protein misfolding. Furthermore, we found out that Exportin-1 (XPO1), a nuclear export carrier protein, is able to shuttle misfolded SOD1 to the cytoplasm as a defence mechanism against toxicity. Consistently, immunoprecipitation assays confirmed direct interaction between misfolded SOD1 and XPO1. In addition, XPO1 levels decreased upon SOD1 knock-down through shRNA, thus suggesting a potential regulatory relationship between these proteins.

2040: **Epigenetic features of C9orf72 gene: DNA methylation and hydroxymethylation as RNA foci modifiers in iPSCs.**

Clara Volpe* (1,2), Claudia Colombrita (1), Patrizia Bossolasco (1), Valentina Gumina (1), Cinzia Tiloca (1), Silvia Peverelli (1), Annamaria Maraschi (1), Donatella Bardelli (1), Vincenzo Silani (1,3), Antonia Ratti (1,2).

1. Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy;
2. Dept. Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan;
3. Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, Milan.

The expansion of the hexanucleotide repeat sequence GGGGCC (>30 repeats) in the first intron of C9orf72 is main genetic cause of ALS and FTD. The 5' CpG island of C9orf72 gene has been found hypermethylated in 30% C9orf72 positive (C9+) ALS/FTD patients compared to the unexpanded patients and healthy controls, suggesting to have a neuroprotective role. This epigenetic trait is detectable in the blood, dermal fibroblasts and brain tissue from the same C9+ individual. Hydroxymethylation, another epigenetic modification with an opposite effect on chromatin condensation compared to methylation, is known to be significantly increased in the nervous system, but it has not been fully investigated in ALS/FTD. Patient-derived iPSCs and differentiated motor neurons (iPSC-MN) represent valuable in vitro models to study disease mechanisms and to test therapeutic options aimed to decrease C9orf72-associated pathological features (RNA foci and DPRs) observed in autoptic brains.

Our work aimed to define the relationship between the epigenetic state of C9orf72 promoter (methylation, hydroxymethylation) and C9orf72-related gene expression and RNA foci formation in iPSCs and iPSC-MNs. We first characterized eight different C9+ iPSC lines, their original fibroblasts and the differentiated iPSC-MNs. We observed that the CpG island epigenetic pattern changed from methylated to unmethylated state and viceversa after iPSC reprogramming, apparently regardless of the original fibroblast's methylation condition, while the epigenetic signature could switch again in iPSC-MNs. Our results also show that methylation state seems to correlate in most cases with a decreased number of RNA foci and to a down-regulation of C9orf72 gene expression of the two pathologic mRNA isoforms (V1 and V3 isoforms), although both hydroxymethylation and repeat expansion length may account for RNA foci number variability.

Our data suggest that patient-derived iPSCs represent a personalized model of C9orf72 pathology where the variable number of druggable RNA foci seems to depend on a complex interplay of epigenetic signatures with opposite effects on C9orf72 repeat expansion transcription.

Session 5: Biomarkers:

2082 Prognostic and disease progression microRNA biomarkers for amyotrophic lateral sclerosis

Iddo Magen* (1), Anna Coenen-Stass (2), Ching-Hua Lu (3), Linda Greensmith (2), Andrea Malaspina (3), Pietro Fratta (2), Eran Hornstein (1).
1 Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.
2 Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK.
3 Centre for Neuroscience and Trauma, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

An unmet need in ALS clinical studies and drug development is prognostic biomarker that will allow subject stratification. An ideal biomarker will be a cell-free, circulating molecule that can be easily measured. The initial value of microRNA biomarkers for amyotrophic lateral sclerosis (ALS) was recently demonstrated by Freischmidt et al. However, it is still unclear whether miRNAs can predict disease course and how they change longitudinally. Here, we performed the largest unbiased miRNA biofluid study, obtaining over 250 samples from the ALS biomarker study. In this context, we further tested a unique cohort of repeated longitudinal measurements, collected over a period of 28 months throughout the disease course. Next generation sequencing (NGS) of small RNA was performed and correlated to available clinical data. In agreement with published works, muscle-enriched miRNA levels are increased in patient samples. The main findings include the discovery of two miRNAs whose levels at baseline predict rapid deterioration and poor prognosis. Two other miRNAs correlate with disease progression and with the levels of plasma TNF-alpha and neurofilament light chain (NFL). In summary, cell-free miRNA emerge as intriguing prognosis and disease progression biomarkers for ALS and the levels of specific miRNAs is able to predict ALS prognosis and might assist to stratify patient groups in clinical trials.
Funding: Motor Neuron Disease Association (MNDA), AFM, TEVA NNE, ISF Legacy, ERA-net

1991:Glucose metabolic patterns along the phenotypic MND-FTD spectrum.

Joke De Vocht* (1,2), Marianne Verhaegen (2), Donatienne Van Weehaeghe (3), Nikita Lamaire (2), Michel Koole (3), Koen Van Laere (3), Philip Van Damme (1,2).
(1) VIB-KU Leuven Center for Brain & Disease Research Leuven, Belgium.
(2) Division of Neurology, University Hospitals Leuven and KU
Leuven, Belgium.
(3) Division of Nuclear Medicine and Molecular imaging, University Hospitals Leuven and KU Leuven, Belgium.

Aims: Previous research highlighted the heterogeneous clinical presentations of Amyotrophic Lateral Sclerosis (ALS). Up to 15% of ALS patients can develop co-morbid frontotemporal dementia, and up to 40% can display cognitive or behavioral impairment. Recent research into Progressive Muscular Atrophy (PMA) and Primary Lateral Sclerosis (PLS) suggested there is also more widespread involvement of the CNS in these clinical phenotypes of ALS. These findings support the hypothesis that classic ALS and FTD may represent phenotypic extremes on an MND-FTD spectrum. The aim of this study is to unravel glucose metabolic patterns characteristic of these clinical phenotypes of ALS.
Methods: 183 ALS patients, 34 PLS patients, 22 PMA patients and 20 healthy controls (HC) underwent [18F]FDG-PET/CT. 124 of these patients were screened with the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) and clustered into four distinct groups according to their ECAS performance: "cognitively normal" (ALS-CN; n= 51), "cognitively impaired" (ALS-Ci; n= 34), "behaviorally impaired" (ALS-Bi/CBi; n=19), and "concomitant FTD" (ALS-FTD; n=18). Static PET images were acquired 30 min post injection of approx. 150 MBq [18F]FDG and statistically analyzed with a voxel-based approach (SPM12).

Comparative analyses were thresholded at Puncorr.< 0.001 and PFWE-corr.< 0.05, kE = 200 voxels at cluster level, unless otherwise specified.
Results: A voxel-wise comparison with HC revealed a continuum of widespread relative bilateral hypometabolism, encompassing frontotemporal and parietal regions when progressing from ALS-CN to ALS-FTD, which remained significant at PFWE-corr.< 0.05. No significant differences were found when comparing ALS-CN to ALS-Ci or ALS-CBi. We also observed relative hypermetabolism in the cerebellar region in all four groups at Puncorr< 0.001, and PFWE-corr.< 0.05 at cluster level.
Moreover, the metabolic profile observed in PLS and PMA appeared highly similar to that of ALS, apart from the absence of significant relative hypermetabolism in the cerebellum in PMA when compared to HC. Prefrontal metabolism was significantly more reduced in PMA and ALS groups when compared to PLS.
Conclusion: These results extend previous findings that [18F]FDG-PET can serve as an imaging biomarker for cognitive and behavioral impairment in ALS. The observed metabolic profile in our PLS and PMA cohort, illustrates the importance of further research on extramotor changes in these patients.

1965: **Prognostic value of neurofilament light chain and C-reactive protein in serum of patients with ALS.**

Maxim De Schaepdryver (1,a,*), Christian Lunetta (2,a), Claudia Tarlarini (2), Lorena Mosca(3), Adriano Chio (4), Philip Van Damme (5,6,7,b), Koen Poesen (1,8,b).
(1) Laboratory for Molecular Neurobiomarker Research, Department of Neurosciences, KU Leuven, Leuven, Belgium.
(2) Neuromuscular Omnicentre (NEMO)-Fondazione Serena Onlus, Milan, Italy.
(3) Medical Genetics Unit, Department of Laboratory Medicine, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy.
(4) ALS Center, 'Rita Levi Montalcini' Department of Neuroscience, University of Turin, Torino, Italy.
(5) Department of Neurology, University Hospitals Leuven, Leuven, Belgium.
(6) Experimental Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium.
(7) Laboratory of Neurobiology, Center for Brain & Disease Research, VIB, Leuven, Belgium.
(8) Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium.
(a) Shared first author-(b) Shared last author.

Objective: Neurodegeneration and neuroinflammation are key hallmarks that characterize the pathophysiology in patients with amyotrophic lateral sclerosis (ALS). The aim of this study was to investigate surrogate serum markers of these hallmarks, namely the neurofilament light chain (NfL) and the C-reactive protein (CRP), to predict the survival of patients with ALS at time of diagnosis.
Methods: A total of 383 patients with ALS were included in this double-center cross-sectional study. Serum NfL and CRP levels were assessed with an electrochemiluminescence assay (Gaiottino J. et al. 2013) and an immunoturbidimetric assay (Roche), respectively. To improve the analysis of the CRP, we will enlarge our cohort of patients with ALS and repeat the measurements using a high sensitive immunoturbidimetric assay (Roche).
Results: The median serum levels of NfL and CRP in patients with ALS were 233 pg/mL (range: 0 – 1476 pg/mL) and 0.2 mg/dL (range: 0.0 – 23.3 mg/dL), respectively. A significant correlation was found between serum levels of NfL and CRP (r = 0.161, 95% Confidence interval (CI) = 0.031 – 0.284; p = 0.012). The univariate survival analysis revealed that high serum levels of NfL or CRP are able to predict an increased risk of death in patients with ALS (NfL: Hazard ratio (HR) = 4.36, 95% CI = 2.89 – 6.59, p < 0.0001; CRP: HR = 2.23, 95% CI = 1.40 – 3.55, p = 0.0007). Next, a multivariate survival analysis was performed including age at onset, disease duration, disease progression rate, forced vital capacity (FVC), C9orf72 mutation carrier, frontotemporal dementia, definite ALS according

to the revised El Escorial criteria, bulbar onset and both biomarkers. In the multivariate analysis, high serum NfL levels, but not CRP levels, predict survival in patients with ALS (HR = 3.18, 95% CI = 1.43 – 7.09; p = 0.0047). In addition, age at onset (HR = 1.03, CI 95% = 1.01 – 1.06; p = 0.0215) and FVC (HR = 0.98, 95% CI = 0.97 – 0.99; p = 0.0030) were able to predict survival as well.
Conclusion: Our findings demonstrate that serum NfL is a prognostic biomarker for patients with ALS. The marker is a strong independent predictor of survival in a multivariate survival model including several other prognostic parameters. Concerning serum CRP levels, the ALS cohort will be enlarged before making any solid conclusion.

1920: **Cervical spinal cord MRI captures presymptomatic pathology in c9orf72 mutation carriers: a longitudinal neuroimaging study.**

(1,2)*Querin Giorgia, MD; (3)Bede P, MD, PhD; (2,4)El Mendili MM, PhD; (2)Li M; (2)Pellegrini-Issac M, PhD; (5,6)Rinaldi D, PhD; (7)Catala M, MD, PhD; (5)Saracino D, MD; (1)Salachas F, MD; (5)Camuzat A, PhD; (2)Marchand-Pauvert V, PhD; (8)Cohen-Adad J, PhD; (5,9,10)Colliot O, PhD; (5,6,11)Le Ber I, MD, PhD; (1,2,12)Pradat PF, MD, PhD; for the Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis (PREV-DEMALS) Study Group.
1-APHP, Département de Neurologie, Centre Référent SLA, Hôpital Pitié-Salpêtrière, Paris, France.
2- Laboratoire d'Imagerie Biomédicale, CNRS, INSERM, Sorbonne Université, Paris, France.
3- Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.
4- Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, USA.
5- Institut du Cerveau et de la Moelle Épinière, Sorbonne Université, INSERM U1127, CNRS UMR 7225, Hôpital Pitié-Salpêtrière, Paris, France.
6- Centre de Reference des Démences Rares ou Précoces, Hôpital Pitié-Salpêtrière, Paris, France.
7- APHP, Département de Neurologie, Hôpital Pitié-Salpêtrière, Sorbonne Université, CNRS UMR7622, INSERM ERL 1156, IBPS, Paris, France.
8- NeuroPoly Lab, Institute of Biomedical Engineering, Polytechnique Montreal, Montreal, QC, Canada; Functional Neuroimaging Unit, CRIUGM, Université de Montreal, Montreal, QC, Canada.
9- Aramis Project Team, Inria Research Center of Paris, Paris, France.
10- Centre pour l'Acquisition et le Traitement des Images, Institut du Cerveau et de la Moelle Épinière, Paris, France.
11- Institute of Memory and Alzheimer's Disease, Center of Excellence of Neurodegenerative Disease, APHP, Département de Neurologie, Centre Référent SLA, Hôpital Pitié-Salpêtrière, Paris, France.
12- Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute Ulster University, C-TRIC, Altnagelvin Hospital, Londonderry, United Kingdom.

Background: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) are neurodegenerative conditions with shared genetic susceptibility and a large portion of familial cases due to c9orf72 gene mutations. Brain imaging studies in asymptomatic c9orf72 carriers have demonstrated white (WM) and grey matter (GM) degeneration before the age of 40.
The objective of this study was to investigate whether cervical spinal cord (SC) degeneration can be detected in asymptomatic c9orf72 hexanucleotide carriers using multimodal quantitative imaging.

Methods: 72 asymptomatic individuals were enrolled in a prospective observational study of first-degree relatives of ALS and FTD patients carrying the c9orf72 mutation and were genetically tested. 40 (C9+) carried the pathogenic repeat expansion. Each subject underwent a 3T cervical SC MRI. Quantitative measures of GM and WM atrophy and DTI parameters were evaluated at baseline and 18 months later. Data were analysed on the total population and in two subgroups composed by subjects younger and older than 40 years of age.

Results: No significant difference was observed between C9+ and C9- subjects younger than 40 years of age regarding morphometric and DTI parameters. At baseline, significant WM atrophy was detected at each cervical vertebral level in C9+ compared to C9- subjects older than 40 years of age (FDR-corrected p-value: C2 vertebral level = 0.013; C2-C3 = 0.006; C3-C4 = 0.009; C4-C5 = 0.017; C5-C6 = 0.013; C6-C7 = 0.014) without associated changes in GM and DTI parameters. At 18-month follow-up, WM atrophy in C9+ subjects older than 40 years was accompanied by significant corticospinal tract fractional anisotropy (FA) reduction compared to C9- subjects (p-value = 0.031). Intriguingly, asymptomatic C9+ subjects older than 40 years with a family history of ALS exhibited significant CST FA reduction on their baseline scans.
Discussion: Cervical SC imaging of c9orf72 hexanucleotide carriers does not detect degeneration in subjects younger than 40 years, but WM atrophy is observed in C9+ subjects older than 40 years of age. While WM atrophy remains stable, progressive pyramidal tract FA reduction can be detected on 18-month follow-up. Neuroimaging in c9orf72 related conditions is a powerful and non-invasive tool to characterise presymptomatic pathological changes and disease propagation patterns. Moreover, SC imaging in c9orf72 may have a future role to predict phenotypic conversion to ALS versus FTD.

2000: **The hypometabolic state is a good predictor of a better prognosis in ALS patients.**

Marina Cattaneo (1), Andrea Lizio (1)*, Nicoletta Guanziroli (2), Ettore Corradi (2), Lucia Greco (1), Valeria Sansone (1,3), Christian Lunetta (1)
(1) Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milan, Italy.
(2) Clinical Nutritional Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy.
(3) Department of Biomedical Sciences of Health, University of Milan, Milan, Italy.

Background: Malnutrition and weight loss are survival unfavourable prognostic factors in patients affected by Amyotrophic Lateral Sclerosis (ALS). Energy Expenditure at Rest (REE) is still argument of discussion in the diet clinical practice of ALS patients, even though different studies have highlighted the presence of hypermetabolism. The literature is lacking of information about the prevalence and consequences of the hypometabolic state in ALS.
Our aim is to evaluate the present knowledge in terms of REE in ALS patients by determining the measured REE (mREE) alterations' prevalence and subsequently to determine the relationship between these characteristics and clinical features and survival.
Patients and Methods: Using indirect calorimetry, we measured the mREE in a cohort of ALS patients with a clinical diagnosis of ALS as defined by El Escorial criteria, referred to the centre between January 2011 and December 2017. The measured values were then compared with the Harris Benedict (HB) predictive equation. All recruited patients were also assessed with a set of specific clinical features and survival data.
Results: Three hundred twelve ALS patients with mean age of 63.11 ± 11.82 and male/female ratio of 1.48 (M/F: 186/126) were recruited. Forty-one percent of ALS patients showed an altered metabolism compared to the HB formula, and this data resulted equally split between hypometabolic (19%) and hypermetabolic (22%) patients. Stratifying our cohort in according to the metabolic condition, hypometabolism resulted significantly associated with a delay in the percutaneous endoscopic gastrostomy (PEG) placement, non-invasive ventilation (NIV) adaptation and tracheostomy (IV) placement, compared to hypermetabolism and normometabolism. Moreover, hypometabolic patients showed a significant longer survival compared to normometabolic (HR: 1.78, p-value: <.01) and hypermetabolic (HR: 2.36, p-value: <.01) patients.

Conclusions: An indirect calorimetry is necessary to evaluate REE in ALS patients because the alterations of metabolism are frequent and the possibility to find hypometabolism or hypermetabolism seems to be similar. In greater detail hypometabolism seems to predict a slower disease progression and an improved survival in comparison to normometabolic and hypermetabolic subjects.

2028: **Quantifying Executive Subdomain Dysfunction in ALS using EEG during the Sustained Attention to Response Task.**

Roisin McMackin(1)*, Stefan Dukic(1,2), Rangariroyashe Chipika(3), Teresa Buxo(1), Antonio Fasano(1), Mark Heverin(1), Richard Reilly(4), Niall Pender(1,5), Peter Bede(3), Muthuraman Muthuraman(6), Bahman Nasseroleslami(1), Orla Hardiman(1,5).
1. Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Dublin, Ireland
2. Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands
3. Computational Neuroimaging Group, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Dublin, Ireland
4. Trinity Centre for Bioengineering, Trinity College Dublin, University of Dublin, Dublin, Ireland
5. Beaumont Hospital, Dublin, Ireland
6. Department of Neurology, Movement disorders and Neurostimulation, Biomedical Statistics and Multimodal Signal Processing Unit, Johannes-Gutenberg-University Hospital, Mainz, Germany.

Background: Executive dysfunction is the most common cognitive impairment in ALS, identified in approximately 25% of patients by psychological tasks [1]. However, such tasks may not detect early cognitive pathology and often capture multiple cognitive subdomains. By recording EEG during these tasks, abnormalities in engaged neural networks can be measured in real-time, including early, preclinical changes. Specific cognitive-behavioural subdomains can be subsequently disentangled by the temporal profiles of their associated measures, while their neural sources can be mapped by source localisation, facilitating a more targeted interrogation. For this purpose, we are recording EEG during the sustained attention to response task (SART), which provides a set of cognitive-behavioural ERPs, including: a parietal P3 peak attributed to processing of task relevant properties; a frontal P3 peak associated with orientation to targets; and a frontal N2 peak which indexes inhibition [2]. These ERPs, therefore, may provide a battery of sensitive biomarkers that quantify several executive subdomain impairments in ALS.Aim: To quantify and localise cortical dysfunction during executive task performance in ALS, using EEG ERPs.
Methods: EEG (128-channel), response time and behavioural accuracy during SART has been recorded from 10 patients and 24 age-matched controls to date. Preliminary analysis includes investigation of changes in behaviour and the N2 and P3 peak amplitudes and LCMV beamforming to localise cortical dysfunction.
Results: Patients demonstrated delayed parietal P3 ($p=0.004$, AUROC=0.844), with source analysis revealing reduced prefrontal, midcingulate and parietal cortex activation during P3 in ALS (FDR=0.1). By contrast, N2, frontal P3, response time and accuracy did not differ between ALS and controls. However, frontal P3 (but not parietal P3) amplitude negatively correlated to response accuracy in both groups ($p=0.0078$).
Discussion: The parietal P3 and frontal P3 responses in the SART can be used as quantitative biomarkers of ALS and ALS cognitive subphenotypes respectively.
References : 1. Phukan et al., JNNP, 83(1), 2012/2. Zordan et al., Brain Cogn., 66(1), 2008.

Session 6 : Disease Mechanisms 2:

1932 : **Molecular and cellular functions of the C9ORF72 protein and its potential implication in ALS.**

Manon Boivin* (1), Véronique Pfister (1), Florine Roussange (2), Cécile Martinat (2), Nicolas Vitale (3), Cédric Raoul (4), Nicolas Charlet-Berguerand (1).
(1) Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch-Graffenstaden, France
(2) Institut des cellules Souches pour le Traitement et l'Etude des maladies Monogéniques (I-Stem), Evry, France
(3) Institut des Neurosciences Cellulaires et Intégratives (INCI), Strasbourg, France
(4) Institut des Neurosciences de Montpellier (INM), Montpellier, France.

An expansion of G4C2 repeats in the first intron of the C9ORF72 gene is the main genetic cause of ALS. Several mechanisms have been proposed to explain the toxicity of these repeats. First, G4C2 repeats are transcribed into toxic RNA that sequester specific RNA binding proteins. Second, G4C2 repeats are translated in toxic Di-Peptide Repeat (DPR) proteins. Third, these repeats promote DNA epigenetic changes at the C9ORF72 promoter 1b, leading to a reduction of C9ORF72 expression. Consequently, individual carrier of a G4C2 expansion present a 40% reduction in C9ORF72 expression. Haploinsufficiency experiments in zebrafish and in iPS cells suggest a potential contribution of C9ORF72 reduction in motor neurons dysfunctions. However, the molecular and cellular roles of the C9ORF72 protein remain to be fully characterized.
C9ORF72 forms a complex with two other proteins, SMCR8 and WDR41. This complex acts as a GDP/ GTP exchange factor for specific RAB GTPases, which are proteins involved in the formation, transport and fusion of cytoplasmic vesicles. In that aspect, C9ORF72 has been found to regulate autophagy, a key catabolic process based on the formation of a vesicle, the autophagosome, around protein aggregates or organelles targeted for degradation. Our objective is to better understand the molecular and cellular functions of the C9ORF72 complex, and notably what vesicle trafficking it does regulate.
We developed novel antibodies against C9ORF72 and SMCR8 as well as C9ORF72 crispr-CAS9 knockout cells. Preliminary results indicate that C9ORF72 is involved in the formation of the autophagosome, notably at the budding of the autophagic vesicles from the endoplamic reticulum. We are now investigating this molecular mechanism and notably which RAB GTPases are regulated by C9ORF72.
In parallel, we also found that both C9ORF72 and SMCR8 co-localize with chromogranin A (CHGA), a marker of Large Dense Core Vesicles (LDCV). LDCV are storage vesicles for hormones and neuropeptides in endocrine and neuro-endocrine cells. Interestingly, we found that siRNA-mediated decrease expression of C9ORF72 leads to reduced levels of CHGA in neuro-endocrine cells. We are now testing the pathological consequences of a decreased expression of C9ORF72 on Large Dense Core Vesicles trafficking in mice and iPS cells.
We hope that these results will help to better understand the function of the C9ORF72 protein and its role in motor neurons dysfunctions in ALS.

2098: **Inclusions of Misfolded wt-SOD1 are common in ALS patients with mutations in C9orf72 and other ALS/FTD-associated genes**.

Karin Forsberg*,1,2 Karin Graffmo,1 Bente Pakkenberg,3 Markus Weber,4 Martin Wirenfeldt,5,6, Stefan L. Marklund,7 Thomas Brännström,1 Peter M. Andersen2.
1. Department of Medical Biosciences, Division of Pathology, Umeå University, Umeå, Sweden
2. Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden
3. Department of Neurology, Research Laboratory for Stereology and Neuroscience,

Bispebjerg-Frederiksberg Hospital Copenhagen, and Institute of Clinical Medicine, Faculty of Health, University of Copenhagen, Copenhagen, Denmark
4. Kantonsspital St. Gallen, Muskelzentrum/ALS Clinic, St. Gallen, Switzerland
5. Department of Pathology, Odense University Hospital, Odense, Denmark
6. Department of Clinical Research, University of Southern Denmark, Odense, Denmark
7. Department of Medical Biosciences, Division of Clinical Chemistry, Umeå University, Sweden.

SOD1WT inclusions. Minute amounts of misSOD1WT inclusions were detected in two of 20 patients deceased from non-neurological causes and in 4 of 10 patients with other neurodegenerative diseases. Comparison was made with 17 patients with 9 different SOD1 mutations: Morphologically, the inclusions in patients with mutations in C9orf72HRE, FUS, KIF5A, NEK1, VAPB and ALSIN resembled inclusions in patients carrying the wt-like SOD1D90A mutation whereas patients carrying unstable SOD1 mutations (A4V, V5M, D76Y, D83G, D101G, G114A, G127X, L144F) had larger skein-like SOD1-positive inclusions.
Conclusion: Abundant inclusions containing misfolded SOD1WT are found in spinal and cortical motor neurons in patients carrying mutations in six ALS-causing genes other than SOD1. This suggests that misfolding of SOD1WT can be part of a common downstream event that may be pathogenic. The new anti-SOD1 therapeutics in development may have applications for a broader range of patients.

1945: **Antisense (C4G2)n RNA repeats from C9orf72 mutation bind RNA binding proteins**.

Mirjana Malnar (1,2), Simona Darovic (1), Maja _talekar (1), Boris Rogelj (1, 3, 4).
1 Department of Biotechnology, Jo_ef Stefan Institute, Ljubljana, Slovenia.
2 Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.
3 Biomedical Research Institute, BRIS, Ljubljana, Slovenia.
4 Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia.

The effects of mutation in C9orf72 gene are still not entirely known, even though the mutation is the main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Sense (G4C2) and antisense (C4G2) RNA transcripts of the mutation are proposed to sequester different RNA binding proteins, therefore impeding their normal cellular functions. Several proteins have been identified so far as the interacting proteins of RNA foci formed by RNA transcripts of mutation. Mostly proteins interacting with sense RNA were identified and their interaction studied, while much less is known about antisense RNA repeats. In our work we focused on antisense repeats. We have set up RNA pull-down assay using long, biologically relevant RNA constructs (32xC4G2). Constructs contained S1m aptamer on one side, which enabled binding of the constructs to the streptavidine magnetic beads. Several proteins have been identified using this method in combination with mass spectrometry. Proteins involved in protein synthesis and cytoskeleton stability were among main interactors. All the proteins were also tested for interaction with sense RNA (48xG4C2) in vitro. Interactions were also tested in cells. We used C9orf72 mutation-positive patient derived fibroblasts, lymphoblasts and iPSNs. We were able to confirm interaction of proteins identified in in vitro experiments with sense and antisense RNA foci in these cells. Further evaluation of these interactions will be important in defining their role for disease progression and development.

1984: **The interactome of human mutant TDP-43 identifies key pathways dysregulated in amyotrophic lateral sclerosis.**

Emily Feneberg (1)*, David Gordon (1), Alexander G. Thompson (1), Philip D. Charles (2), Ruxandra Dafinca (1), Imre Mäger (3), Matthew Wood (3), Roman Fischer (2), Benedikt M. Kessler (2), Elizabeth Gray (1), Martin R. Turner (1), Kevin Talbot (1).
(1) Nuffield Department of Clinical Neurosciences, University of Oxford, UK.
(2) Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, UK.
(3) Department of Paediatrics, Medical Sciences Division, University of Oxford, UK.

Background: The C-terminal region of TDP-43 is important for protein-protein interactions Using a BAC-transgenic mouse model, we therefore sought to study the effect of a mutation (M337V) in this region in disrupting the TDP-43 interactome to give insights into ALS pathogenesis and identify pathways which are potential therapeutic targets.
Method: Affinity-enrichment mass spectrometry analysis for the interactome of (BAC)-transgenic human Ypet-tagged wild-type or M337V mutant TDP-43 was conducted from mouse motor neuron cultures. Specific interactors were determined by label-free quantification. Dysregulated pathways were identified using Gene Ontology enrichment analysis. Co-immunoprecipitation and immunohistochemistry for Poly(A)-binding protein (PABPc) with semi-automated analysis of stress granules was performed. Extracellular vesicles were extracted from motor neuron conditioned media using ultrafiltration liquid chromatography and quantified by nanoparticle tracking analysis.
Results: Specific interactors of human wild-type TDP-43 were enriched for transcription, translation and poly(A)-RNA binding. In response to oxidative stress wild-type TDP-43 interactions were enriched for proteins in the endoplasmic reticulum, calcium binding and endosomal transport pathways. The TDP-43M337V interactome had a significant decrease in wild-type interactors (p=0.036) and showed acquired interactions with proteins of the ubiquitin-proteasome and lysosome, both of which have been independently linked to ALS pathogenesis. Importantly, binding of human wild-type TDP-43 to PABPc, which is involved in translation and formation of stress granules, was shown to be disrupted in the presence of the C-terminal pathogenic mutation and confirmed in human cellular models. This was linked to a decreased number of M337 mutant TDP-43 motor neurons with PABPc positive stress granule formation (p=0.004) and a decreased number and average area of stress granules per neuron (p=0.0192, p=0.013). Gene Ontology enrichment analysis further suggested that mutant TDP-43 altered binding to proteins involved in extracellular secretion such as rab-GTPases associated with reduced extracellular vesicle secretion in M337V mutant mouse and human iPSCs-derived motor neurons.
Conclusion: Analysis of the interactome of mutant TDP-43 reveals disruption of specific cellular pathways that may be further explored for therapeutic targeting.

2009: **Role of potential cell-to-cell transmission of dipeptide repeat proteins in C9ORF72 ALS/FTD pathology.**

*Marchi, P.(1), Bousset, L. (2), Brasseur, L. (2), Ferraiuolo, L. (1), Hautbergue, G. (1), Melki R. (2), Azzouz, M. (1)
(1) Sheffield Institute for Translational Neuroscience, Sheffield University, Sheffield, UK
(2) CEA, Institut François Jacob (MIRcen) and CNRS Laboratory of Neurodegenerative Diseases (UMR9199), Fontenay-aux-Roses, France.

A GGGGCC hexanucleotide repeat expansion within the C9ORF72 gene is the most common genetic cause of both amyotrophic lateral sclerosis and frontotemporal dementia. Sense and antisense repeat-containing transcripts undergo repeat-associated non-AUG translation to produce toxic dipeptide repeat proteins (DPRs).
In many neurodegenerative diseases, the progression of pathology is driven by proteinaceous aggregates deriving from misfolded proteins that self-aggregate and undergo

'prion-like propagation' throughout the CNS by seeding the misfolding of the endogenous conformers.
In this project, we aim to investigate the potential occurrence of prion-like propagation of C9ORF72 DPRs in vitro. To test this, we first address whether cells can take up DPRs once added to the medium and then transfer these proteins from cell-to-cell of the same culture or in a co-culture system leading to seeding effects. E. coli-derived DPRs (poly-GA and poly-PA) were produced from our collaborator Dr Melki and were fluorescently-labelled and tagged.
Our findings show that human astrocytoma and iAstrocytes from C9ORF72 patients can take up poly-GA DPRs from the medium. DPR clustering and internalisation occur both in the nucleus and in the cytoplasm.
Live-imaging shows that poly-GA and poly-PA DPRs have a circular shape and can move from cell to cell in the same culture through tunneling nanotubes-like structures. Moreover, DPR-lysosome co-localization is revealed by ICC and the number of lysosomes increases in cells treated with poly-GA soluble DPRs, indicating that the aggregate burden might produce changes in lysosome cell biology and biogenesis. The DPR-lysosome interaction will be further explored by super-resolution STORM imaging, whose resolution power can also provide more detail on both clustered and diffused distribution of DPRs on the plasma membrane of cells.

1943 **Behavioral deficits, TDP-43 pathology and progressive neuropathology in Cyclophilin A knock-out mice: A mouse model of Frontotemporal Dementia**.

Laura Pasetto* (1), Silvia Pozzi (2), Edoardo Micotti (3), Milica Cerovic (3), Mirjana Carli (3), Gianluigi Forloni (3) and Valentina Bonetto (1).
(1) Department of Biochemistry and Molecular Pharmacology, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milano (Italy).
(2) CERVO Brain Research Center, Laval University, Québec City, Québec, Canada.
(3) Department of Neuroscience, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milano (Italy).

Cyclophilin A (PPIA) is a multifunctional protein abundantly expressed in the central nervous system. PPIA has been associated with different human diseases, but its role in pathogenesis is still unknown. We first associated PPIA with nervous system degeneration, identifying it as a translational biomarker of ALS with a protective role in ALS pathology.
We recently demonstrated that PPIA is a functional interacting partner of TDP-43 and regulates its nuclear-cytoplasmic trafficking. TDP-43, a nuclear protein involved in RNA processing, is the major component of neuronal cytoplasmic inclusions in frontotemporal lobar degeneration patients (FTLD-TDP). The molecular mechanisms at the basis of TDP-43 pathology have not been elucidated yet. Here we proposed PPIA as a major player of this process.
We show that PPIA knock-out (PPIA-/-) mice recapitulate major features of FTLD-TDP, such as aggregation and mislocalization of TDP-43 in the brain. PPIA-/- mice show also progressive atrophy of cortex and hippocampus, with neuronal loss. In absence of PPIA, mice exhibited an initial increased disinhibition and sociability that turned into reduced social interest later in the pathology with no memory and motor impairment, reminiscent of the human condition. Finally, we demonstrated that reduction of PPIA is associated with low levels of progranulin, the major genetic cause of FTLD.In conclusion, our findings indicate that PPIA has an important effect on neuronal survival and its depletion results in FTLD-related deficits. Moreover, PPIA-/- mouse represents a useful animal model to understand the molecular mechanism behind TDP-43 pathology and its involvement in FTLD-TDP.

Session 7: Therapeutics:

2081: **Safety, PK, PD, and exploratory efficacy in single and multiple dose study of a SOD1 antisense oligonucleotide (BIIB067) in participants with ALS.**

Timothy Miller* (1), Merit Cudkowicz (2), Pamela Shaw (3), Danielle Graham (4), Stephanie Fradette (4), Hani Houshyar (4), Frank Bennett (5), Roger Lane (5), Ivan Nestorov (4), Laura Fanning (4), Ih Chang (4), Toby Ferguson (4).
(1) Washington University School of Medicine, Neurology Department, St. Louis, MO 63110, USA.
(2) Healey Center, Mass General Hospital, Boston, MA, USA.
(3) Sheffield Institute for Translational Neuroscience, and NIHR Sheffield Biomedical Research Centre, University of Sheffield, Sheffield, UK.
(4) Biogen, Cambridge, MA, USA.
(5) Ionis Pharmaceuticals, Carlsbad, CA, USA.

Objective: To evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of an antisense oligonucleotide (BIIB067) designed to reduce superoxide dismutase (SOD1) mRNA in people with amyotrophic lateral sclerosis (ALS) with SOD1 gene mutation (SOD1-ALS).
Background: ALS is a fatal, neurodegenerative disease characterized by loss or dysfunction of upper and lower motor neurons. Approximately 2% of ALS cases are linked to SOD1 mutations. Over 200 SOD1 mutations have been identified with substantial variation in rate of disease progression. Toxicity of mutant SOD1 is secondary to gain of function, not loss of SOD activity, suggesting SOD1 reduction may be therapeutic. BIIB067 is under development for treatment of SOD1-ALS.
Methods: This randomized, placebo-controlled, single- and multiple-ascending dose (SAD/MAD) study enrolled participants with ALS. In the MAD portion of the study, 50 participants with confirmed SOD1 mutation were randomized (3:1 BIIB067:placebo) to receive BIIB067 (20, 40, 60, or 100 mg) or placebo for 12 weeks. Safety (primary), PK/PD (secondary), and efficacy (exploratory) were assessed.
Results: The majority of adverse events (AEs) were mild or moderate in severity. Dose-dependent increases in BIIB067 concentrations in plasma and CSF were observed. A statistically significant reduction of CSF SOD1 was observed in the 100-mg cohort (n=10) versus placebo (n=12) (p=0.002) and suggested substantial reduction of CNS tissue SOD1. Lowering of CSF phosphorylated neurofilament heavy and slowing of functional decline as measured by ALS Functional Rating Scale Revised scores, slow vital capacity, and muscle strength were observed in the 100 mg cohort versus placebo. In participants with SOD1 mutations known to be rapidly progressive, a greater difference between the 100-mg and placebo groups was observed across these measures compared to those with other mutations.
Conclusions: This first report of BIIB067 in SOD1-ALS demonstrates reduction of SOD1 in CSF and strongly supports further investigation of BIIB067 efficacy in people with SOD1-ALS.
Study supported by: Biogen.

2029: **Results of FORTITUDE-ALS: A Phase 2, Double-blind, Randomized, Placebo-Controlled, Study to Evaluate Efficacy, Safety and Tolerability of Reldesemtiv.**

Jeremy M Shefner(1)(*), Jinsy A Andrews (2), Angela Genge (3), Carlayne Jackson (4), Noah Lechtzin (5), Timothy M Miller (6), Bettina M Cockroft (7), Fady I Malik (7), Lisa Meng (7), Jenny Wei (7), Andrew A Wolff (7), Stacy A Rudnicki (7), and the FORTITUDE-ALS Study Group.
(1) Barrow Neurological Institute, Phoenix, AZ, USA.
(2) Columbia University, New York, NY, USA.
(3) Montreal Neurological Institute and Hospital, Montreal, QC, Canada.
(4) University of Texas Health Science Center, San Antonio, TX, USA.

(5) Johns Hopkins School of Medicine, Baltimore, MD, USA.
(6) Washington University School of Medicine, St. Louis, MO, USA.
(7) Cytokinetics, Inc., South San Francisco, CA, USA.

Objective: To evaluate safety, tolerability, and preliminary efficacy of reldesemtiv in patients with amyotrophic lateral sclerosis (ALS).
Background: Reldesemtiv (CK-2127107), a selective small molecule fast skeletal muscle troponin activator, sensitizes the sarcomere to Ca2+. Reldesemtiv increased the force generated by the tibialis anterior muscle vs. Placebo in response to nerve stimulation in healthy volunteers. Derived from a different chemical scaffold, designed to limit crossing of the blood-brain barrier, it is expected to have fewer central nervous system adverse effects than tirasemtiv, a similar though less potent compound.
Methods: Key inclusion criteria for FORTITUDE-ALS were diagnosis within 24 months and upright slow vital capacity (SVC) ≥60% of predicted. Participants were randomized 1:1:1:1 to placebo or reldesemtiv at 150 mg twice daily (BID), 300 mg BID, or 450 mg BID for 12 weeks. Primary endpoint was change from baseline (BL) in SVC at 12 weeks. Secondary outcome measures were quantitative strength testing using hand-held dynamometry and the ALS Functional Rating Scale-Revised (ALSFRS-R). Exploratory outcome measures were weekly SVC performed at home, and two application-based evaluations: one of fine motor skills in clinic, and the other involving voice recording at home and in clinic. Safety and pharmacokinetic endpoints were also captured. Patients were enrolled in the USA, Canada, Australia, Ireland, Spain, and the Netherlands.
Results: FORTITUDE-ALS completed enrollment in November 2018, with the last patient's last visit in March 2019. Of 605 patients screened, there were 147 screen failures; SVC below the minimum required was the most common cause. One randomized patient withdrew consent prior to the Day 1 visit. Of 457 treated, 258 patients were on riluzole, 19 on edaravone, 94 on both, and 86 on neither. Mean age was 58.7+10.7 years, 277 (60.6%) were male, and 423 (92.6%) white. Mean time since onset of symptoms was 22.8 months and mean time since diagnosis was 8.6 months. Site of onset was bulbar in 87 patients, upper extremity in 201 patients, and lower extremity in 169 patients. A negative family history of ALS was reported by 387 (84.7%) patients. Mean BL percent predicted SVC was 84.7% and average BL ALSFRS-R total score was 37.4. Top line efficacy, safety, and tolerability data will be presented.
Conclusions: The results of this study will determine whether phase 3 studies of reldesemtiv should be conducted in ALS.

1939**: The REFALS-ES open-label extension study of oral levosimendan (ODM-109).**

Merit Cudkowicz (1), Angela Genge (2), Nicholas Maragakis (3), Susanne Petri (4), Leonard van den Berg (5), Valtteri Aho (6), Chris Garratt (7), Toni Sarapohja (8), *Ammar Al-Chalabi (9).
1. Massachusetts General Hospital, Boston, USA.
2. Montreal Neurological Institute and Hospital, Montreal, Canada.
3. Johns Hopkins University, Baltimore, USA.
4. Medizinische Hochschule Hannover, Hannover, Germany.
5. University Medical Center Utrecht, Utrecht, The Netherlands.
6. Orion Pharma, Orion Corporation, Turku, Finland.
7. Orion Pharma, Orion Corporation, Nottingham, UK.
8. Orion Pharma, Orion Corporation, Espoo, Finland.
9. King's College London, London, UK.

Levosimendan is a calcium sensitising drug that binds to Troponin C and improves force generation in both cardiac and skeletal muscle, without increasing oxygen consumption. Intravenous levosimendan (Simdax®) has been used to treat acute heart failure since 2000 and is approved in nearly 60 countries worldwide. Levosimendan was shown to improve

diaphragm neuromechanical efficiency in healthy volunteers and to have positive effects on supine slow vital capacity (SVC) in the LEVALS pilot study in patients with amyotrophic lateral sclerosis (ALS). The clinical effects of oral levosimendan are being confirmed in the ongoing REFALS phase 3 trial (NCT03505021) in patients with ALS. REFALS-ES is an open-label extension study for patients who complete the REFALS study.
The REFALS study is enrolling 450 adult patients with definite, probable or laboratory supported probable ALS and some degree of respiratory dysfunction (sitting SVC 60-90%) at 104 clinical sites in 14 countries in EU, USA and Australia. Randomised patients are treated with oral levosimendan (target dose 2mg daily) or placebo for 48 weeks in a double-blind, parallel group design. The primary endpoint is supine SVC, while secondary endpoints include ALSFRS-R adjusted for patient survival, the occurrence of respiratory events, clinical global impression, Borg scale for dyspnoea and scales assessing sleep and sleepiness. The safety profile of oral levosimendan is being assessed using standard measures including adverse events, vital signs, 12-lead electrocardiogram and laboratory safety tests at regular intervals throughout the 48 weeks treatment period. Potential impacts on the level and costs of care are recorded by patients in a diary.
All patients who complete 48 weeks of dosing in the REFALS study will be eligible to enter the REFALS-ES study, provided no new safety exclusion has arisen. In addition to providing continued treatment for REFALS patients, the study will generate important long-term safety and efficacy information during more usual care. Following completion of all REFALS assessments, patients will be treated with levosimendan (target dose 2mg/day) for as long as clinically required, with periodic assessments including SVC, ALSFRS-R and patient safety, in a flexible visit structure combining on-site and telephone assessments. The first patient is expected to join the study in June 2019. Details of the REFALS-ES study design and objectives will be presented.

2039: **Long-term Outcome of Filgrastim (G-CSF) in ALS Patients in Reference to PRO-ACT.**

S. Johannesen (1)*, B. Budeus (2), T-H. Bruun (1), S. Peters(1), I. Kobor(1), W. Schulte-Mattler (1), S. Iberl (5), A. Schneider (2), W. Koch (3), V.C.Samara (4), T. Pukrop (5), R. Huie (6), A. Ferguson (6), U. Bogdahn (1).
1: Department of Neurology, University of Regensburg, Germany.
2: Life.Data.Science, Heidelberg, Germany.
3: BDS Koch, Schwetzingen, Germany.
4: Stanford Neuroscience Health Center, Palo Alto, California, USA.
5: Department of Hematology, University of Regensburg, Germany.
6: Department of Neurosurgery, UCSF, San Francisco, California, USA

Background: We investigated the outcome of long-term G-CSF treatment in ALS patients.
Methods: 36 definite ALS patients (mean age 52 yrs, mean ALSFRS-F 38.6/48, 70.3% m, 29.7% f) were treated with informed written consent on a named outpatient basis from 1/2010 to 3/2017 (mean dose: 464 Mio IU G-CSF/ month, median treatment duration 13.7 months). Survival and disease progression (ALSFRS-R) matching were referenced to the PRO-ACT data base (10.700 patents, mean age 55.6 yrs, mean ALSFRS-R 38.4/48, 62.4% m, 37.6% f). A survival prediction model based on PRO-ACT was established
Results: Safety, feasibility and tolerance were excellent. Significant differences in ALSFRS-R slopes between G-CSF and PRO-ACT depended on time between first symptom and treatment onset: in patients with less than 10 months disease duration G-CSF treated patients lost 3.73 ALSFRS-R points less than PRO-ACT-patients at 6 months. Profiles of ALSFRS-R in the G-CSF group presented flattening compared to the PRO-ACT group over 36 months.

Multi parameter adjusted survival analyses revealed a significant superiority for G-CSF treatment, specifically compared to the best PRO-ACT subgroup of riluzole and placebo treated patients. This benefit was confirmed with matched pairs in both overall PRO-ACT and the riluzole and placebo subgroup: mean survival in G-CSF vs. PRO-ACT was 594 vs. 373 days, and 596 vs. 403 day in G-CSF vs. riluzole and placebo subgroup.
A model for individual survival estimation was created based on patient variables from deceased patients and validated within the PRO-ACT. Applying the model to G-CSF treated patients revealed significantly longer observed than model predicted survival times. A highly G-CSF-responsive subgroup (N=15) with a distinct biomarker profile was delineated, with a 3-fold life expectancy. After 3 months of G-CSF-treatment, a biomarker profile could already predict response / non-response accurately.
Conclusions: Long-time G-CSF therapy in ALS patients seems extremely encouraging and safe. A survival prediction model and a matched pair analysis indicated survival times significantly in favor of G-CSF treatment compared to PRO-ACT database. G-CSF treated patients presented a slower decline in ALSFRS-R already over the first 6 months and a further flattening of the ALSFRS-R slope was present after 2 and 3 years. G-CSF responding patients with longer than model-predicted survival also had distinct biomarker profiles.

2002: **Gene editing as a potential therapeutic approach for ALS/FTD-associated with expanded C9ORF72.**

João Alves-Cruzeiro*(1), Claudia Bauer(1), Ian Coldicott(1), Guillaume Hautbergue(1), Monika Myszczynska(1), Laura Ferraioulo(1), Mimoun Azzouz(1).
(1)-Sheffield Institute for Translational Neuroscience, Sheffield, United Kingdom.

A hexanucleotide repeat expansion (GGGGCC or G4C2) in the first intron of chromosome 9 open reading frame 72 (C9ORF72) is the most common genetic cause for Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Despite being two different neurodegenerative diseases, they have common molecular hallmarks such as the presence of RNA Foci and Dipeptide repeats (DPRs) derived from the transcription and translation of the expansion sequence, respectively. Despite decades of research, the treatment of FTD still relies on symptoms' management while Riluzole and Edaravone remain the only disease-modifying treatments currently approved for ALS, with very modest effects on disease progression and survival, highlighting the need for more targeted and efficient therapeutic strategies.
It was in this context that we proposed to develop and test a new experimental treatment, consisting of a highly targeted gene editing strategy, using the CRISPR-Cas9 tool. Our approach focuses on expressing SaCas9 through an AAV delivery vector together with a pair of gRNAs that cut in the flanking regions of the hexanucleotide expansion, excising the repeats and allowing the re-ligation of the gene.
We successfully cloned Staphylococcus aureus Cas9 and two U6-driven gRNAs in a single AAV expression vector and demonstrated the efficacy of our strategy in a human cell line, achieving robust gene editing without affecting the expression of WT C9ORF72 protein. To test our approach in cells with a pathogenic expansion, we produced AAV viruses and transduced a line of induced Astrocytes derived from patient's fibroblasts and cortical neurons derived from a BAC mouse model of expanded C9ORF72. In both models, transduction with our therapeutic virus was successful in editing the C9ORF72 gene by excising the full expansion and reducing RNA Foci expression and poly(GP) DPRs. Our proof-of-concept study might provide the basis for a more targeted and efficient therapy for C9ORF72-ALS/FTD than what is currently available.

Session 8: Precision Medicine:

1902: **Using Artificial Intelligence to Uncover Novel Drug Therapies and Targets for Amyotrophic Lateral Sclerosis (ALS).**

Monika Myszczynska*(1), Matthew Stopford (1), Nora Markus (1), Mark Rackham (2), Richard Mead (1), Laura Ferraiuolo (1).
1: Sheffield Institute for Translational Neuroscience (SITraN), Department of Neuroscience, University of Sheffield, Sheffield, United Kingdom
2: BenevolentAI, London, United Kingdom.

Background: Although ALS is characterised by motor neuron (MN) death, astrocytes have been implicated in the neurodegenerative process. There are currently no disease course-altering drug therapies available for ALS. Developments in the field of artificial intelligence (AI) have shown a potential to uncover novel therapies for ALS in a short space of time, as AI algorithms can identify links between a disease and potential beneficial compounds after a thorough scan of the scientific literature, omics databases, chemical libraries and other public sources. One such effort by a biotechnology company BenevolentAI identified two cancer drugs, gefitinib and Compound A, as having repurposing opportunities for ALS.
Aim of the study is to determine the effect of gefitinib and Compound A on MN survival and decipher their modes of action in ALS patients and healthy controls in pathophysiologically relevant in vitro models, Method: C9orf72-ALS, sporadic ALS, and control induced astrocyte (iAstrocyte) lines (n=3 per group) were plated onto 384-well plates, pre-treated with gefitinib and Compound A and used for co-culture screening. Protein levels of their known targets were measured. Cells were subsequently assessed for levels of TDP-35 fragments, which was also measured in induced neurons (iNeurons) obtained from the same cell lines. Autophagy function assay was performed in both iAstrocytes and HEK293 cell lines to determine the drugs' ability to activate autophagy.
Results and conclusions: Both gefitinib and Compound A treatment led to a significant ($p<0.05$) rescue of MN survival on ALS iAstrocytes compared to 12 initial candidates. Consistently, western blotting analysis has shown gefitinib treatment to significantly ($p<0.05$) reduce the levels of soluble TDP-43 fragments, a hallmark of ALS pathology, in patient iAstrocytes and iNeurons. Autophagy assays showed gefitinib to be an activator of autophagy, suggesting that activation of this pathway contributes to MN survival and clearance of soluble TDP-35 fragments found in protein aggregates. Interestingly, while showing a significant improvement in MN survival, Compound A had no effect on TDP-43 fragmentation, indicating that the two compounds exert their therapeutic effects through different mechanisms, which we are currently investigating through functional assays and RNA-sequencing.
In conclusion, our data so far prove that AI is an upcoming promising approach for drug discovery.

2100: **Preliminary Evidence of New ALS Phenotypes based on Clustering of Spectral EEG Measures.**

Stefan Dukic (1,2)*, Roisin McMackin (1), Teresa Buxo (1), Antonio Fasano (1), Rangariroyashe Chipika (3), Marta Pinto-Grau (1), Emmet Costello (1), Christina Schuster (3), Michael Broderick (4), Parameswaran M. Iyer (1), Kieran Mohr (1), Amina Coffey (1), Brighid Gavin (1), Mark Heverin (1), Niall Pender (1), Peter Bede (3), Muthuraman Muthuraman (5), Bahman Nasseroleslami (1), Edmund C. Lalor (4, 6, 7), Orla Hardiman (1,6,8)
1 Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Ireland.
2 Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands.

3 Computational Neuroimaging Group, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Ireland.
4 Trinity Centre for Bioengineering, Trinity College Dublin, University of Dublin, Ireland.
5 Department of Neurology, Movement disorders and Neurostimulation, Biomedical Statistics and Multimodal Signal Processing Unit, Johannes-Gutenberg-University Hospital, Mainz, Germany.
6 Trinity College Institute of Neuroscience, Trinity College Dublin, University of Dublin, Ireland.
7 Department of Biomedical Engineering and Department of Neuroscience, University of Rochester, USA.
8 Beaumont Hospital, Dublin, Ireland.

Background:
Amyotrophic lateral sclerosis (ALS) is a progressive heterogeneous neurodegenerative disease affecting both motor and non-motor networks and manifesting as distinct clinical subphenotypes. To date, subphenotyping has been primarily based on the ALSFRS - which is a semi-quantitative clinical measure of motor dysfunction with floor and ceiling effects. An alternative approach is classification of electroencephalography (EEG)-based measures that can quantitatively capture the abnormal patterns of network disruption in ALS [1] underpinning heterogeneous clinical manifestations.

Aim:
To define subphenotypes of ALS using data-driven clustering according to spectral power and whole-brain resting-state functional connectivity.

Methods:
Resting-state EEG was used to estimate spectral power, co-modulation and synchrony in 90 brain regions in 92 ALS patients. Additionally, ALSFRS-R and ECAS subscores were assessed from 83 and 76 patients, respectively. Using canonical correlation analysis (CCA), we defined a low-dimensional space of combined EEG measures that correlate with clinical data [3], in which we applied two clustering methods (k-means and linkage). An optimal number of phenotypes (range 2-10) was determined using two criterions (Calinski-Harabasz and Silhouette).

Results:
CCA identified 3 statistically significant combinations of EEG measures that were used for clustering. Clustering methods reliably defined 4 subphenotypes (S) with distinct clinical features: S1 (low rate of progression, long survival), S2 (low rate of progression, average survival), S2&4 (high rate of progression, short survival). Additionally, S1 showed dominat cognitive, S2 strong motor and cognitive, S3 mild motor and cognitive, and S4 dominant motor impairments.

Discussion:
This study paves the way for development of classification systems based on objective neurophysiological measures that capture both motor and cognitive network dysfunction. This methodology has the potential to provide clinically meaningful subphenotypes that capture aspects of the underlying pathobiology of disease spread. Such a subphenotyping process could be of value in a precision medicine approach to new clinical trials.

1. B. Nasseroleslami et al, Cereb. Cortex, 2017
2. A.T. Drysdale et al, Nat. Med., 2016

2007: **The distinct phenotypical traits of the UNC13A polymorphism in amyotrophic lateral sclerosis.**

Harold Tan*(1), Henk-Jan Westeneng(1), Hannelore van der Burgh(1), Leonhard Bakker(1,2), Kevin van Veenhuijzen(1), Kristel van Eijk(1), Jan Veldink(1), Leonard van den Berg(1).
(1) Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands.
(2) Center of Excellence for Rehabilitation Medicine, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht University and De Hoogstraat Rehabilitation, Utrecht, The Netherlands.

Objective: The rs12608932 single nucleotide polymorphism (SNP) in the UNC13A gene is associated with both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) susceptibility, and may underlie differences of treatment response in clinical trials. Within ALS patients, we aimed to extensively characterise the clinical, cognitive, behavioural, and neuroimaging phenotype of this SNP in UNC13A.
Methods: Using a population-based cohort, we studied 2216 ALS patients without a C9orf72 gene mutation to identify clinical characteristics associated with the UNC13A SNP. A subgroup of 428 ALS patients was used to study cognitive and behavioural profiles using the Edinburgh Cognitive and behavioural ALS screen and the ALS-FTD questionnaire. Neuroimaging characteristics were analysed in 375 patients. All associations were analysed using an additive model. Linear and logistic regression were used to analyse clinical characteristics and behaviour. Survival was analysed using a Cox proportional hazards model corrected for the linear predictor of the ENCALS survival model. ECAS cognitive scores were analysed using Poisson regression, corrected for age, gender and education. Neuroimaging parameters were corrected for age and gender in a linear model.
Results: Genotyping of the rs12608932 SNP resulted in 1021 A/A, 1208 A/C and 437 C/C genotypes. The C allele of UNC13A was associated with a higher age at symptom onset (median in years A/A 63.5, A/C 65.6, C/C 65.5, $p<0.001$) , more frequent bulbar onset of symptoms (A/A 29.6%, A/C 31.8%, C/C 43.1%, $p<0.001$), higher incidences of ALS-FTD at diagnosis(A/A 4.3%, A/C 5.2%, C/C 9.5%, $p=0.003$), a lower forced vital capacity (median percentage points A/A 92, A/C 90, C/C 86.5, $p<0.001$) and a shorter survival (median in months A/A 33.3, A.C 30.7, C/C 26.6, $p<0.001$). UNC13A was also associated with lower scores on ALS-specific domains ($p=0.023$) and more frequent behavioural impairment ($p=0.045$). In neuroimaging, UNC13A showed more cortical thinning in the left inferiortemporal ($p=0.045$) and the right fusiform cortex ($p=0.036$).
Interpretation: In a large cohort we show that distinct clinical characteristics are found over the genotypes of the rs12608932 SNP in UNC13A. Moreover, cognitive, behavioural, and neuroimaging analyses all support evidence that link UNC13A to the ALS-FTD phenotype. We conclude that UNC13A is an important genotype to take into account when analysing the ALS syndrome.